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A Generic Redelimitation of the *Ionaspis-Hymenelia* Complex (Lichenized Ascomycotina)

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ABSTRACT. A review of the North American taxa within the *Ionaspis-Hymenelia* complex and a cladistic analysis of morphological-anatomical data and enzyme electrophoresis data have revealed delimitations at the generic level that differ from those currently in use. For the first time, *Ionaspis* is defined here on characters other than the nature of the photobiont; it now encompasses the *suaveolens* group (including the type species of *Ionaspis*, *I. chrysophana*), the *odora* group, the *lacustris* group, and the *alba* group. Under this new classification, *Hymenelia* includes the *epulotica* group (which includes the type species of *Hymenelia*, *H. prevostii*), the *haematina* group, and the *melanocarpa* group. The status of *Aspicilia* was not changed in this study, because of the lack of appropriate data; therefore, it is still classified within the Hymeneliaceae. The genus *Eiglera* and the monotypic family Eigleraceae were established previously only on the basis of the presence of an amyloid ascus tip structure and shape of paraphyses. *Eiglera* is subsumed here as part of the Hymeneliaceae because both morphology and allozyme data strongly support a close phylogenetic relationship with *Hymenelia*. This result also questions the principle that families and genera of Ascomycotina must contain elements having the same ascus type. A new method for coding continuous characters is described and applied to the anatomical and morphological data to help solve the phylogenetic relationships within the *Ionaspis-Hymenelia* complex.

Ionaspis Th. Fr. and *Hymenelia* Kremp. are saxicolous crustose lichens found mostly in Arctic-alpine regions (Magnusson 1933; Poelt and Vězda 1981). Some taxa are endolithic and several are aquatic. The only monograph to consider either genus of this complex is Magnusson's (1933) treatment of *Ionaspis*, which is restricted almost entirely to the European representatives. Magnusson's species concept is considered unreliable (Weber 1962, 1968) for the following reasons: 1) some species were based primarily on substrate characteristics; 2) several described species were based on single specimens, and 3) some morphologically variable species were fragmented into several varieties and forms based on environmentally sensitive characters such as the color of the thallus. Moreover, Magnusson (1933) did not include varieties and forms in his key, thereby promoting misidentifications. Recently, Jørgensen (1989) revised *Ionaspis* and published a key for Scandinavia. Also, three new species were described by Jørgensen and Santesson (1989): *I. fuegensis* P. M. Jørg. & R. Sant. from Argentina, *I. granvina* P. M. Jørg. & R. Sant. from Norway, and *I. ventosa* P. M. Jørg. & R. Sant. from Sweden. Although *Hymenelia* was established by Krempelhuber in 1852, Poelt and Vězda (1981) published the first key to the species of *Hymenelia*, based on European material.

The genera *Ionaspis* and *Hymenelia* were always distinguished solely by their different photobionts. Theodor Fries (1871) segregated *Ionaspis* from *Aspicilia* A. Massal. based only on its having *Trentepohlia* Mart. rather than *Trebouxia* Puym. as a photobiont. The previously described genus, *Hymenelia*, which contained essentially the same group of taxa as *Ionaspis* (under different epithets, and not based on algal differences) was either overlooked or ignored by Th. Fries. Zahlbruckner (1928, 1934) accepted *Ionaspis*, but subsumed *Hymenelia* under *Le-*

canora Lindau sect. *Aspicilia* Stizenb. *Hymenelia* lay forgotten in synonymy until Eigler (1969) resurrected the name for the "Coerulea-Gruppe," and Poelt and Vězda (1981) used the genus in a broader sense to accommodate *H. prevostii* (Duby) Kremp., *H. lacustris* (With.) M. Choisy, and other related taxa.

Eigler (1969) showed that some taxa of *Ionaspis* are more similar to some species of *Hymenelia* than to congeneric species. He also demonstrated that some taxa were too different from the rest of the species in this complex to remain classified within *Ionaspis* or *Hymenelia*. The heterogeneity within this complex is due, in part, to the paucity of readily observable characters. Almost any aquatic lichen having small cryptolecanorine apothecia (i.e., with an excipulum thallinum and apothecial disk embedded in the thallus) was usually classified within either *Ionaspis* or *Hymenelia*, depending on its photobiont. As a result, the distinction between *Ionaspis* and *Hymenelia* based on photobiont differences was increasingly questioned by many lichenologists (Magnusson 1933; Eigler 1969; Ozenda and Clauzade 1970; Wirth 1980; Poelt and Vězda 1981; Hafellner 1984; Clauzade and Roux 1985; Jørgensen 1989).

Two main problems at the generic level have been associated with the taxonomy of the *Ionaspis-Hymenelia* complex: 1) the photobiont difference is the only diagnostic character between these two genera, and 2) intrageneric heterogeneity, in which some taxa are more similar to taxa of the other genus than to congeneric taxa, and with some species seemingly only distantly related to the rest of the species within the complex. The first goal of this study was to delimit distinct homogeneous groups within the *Ionaspis-Hymenelia* complex and to determine their phylogenetic relationships, as well as their affinities with the genus *Eiglera* Hafellner, another genus having cryptolecanorine apothecia. The second goal was to re-evaluate the importance of the photobiotic difference as a diagnostic character between *Ionaspis* and *Hymenelia*. The third goal was to define a new classification of the hymenelioid lichens at the generic level, including *Eiglera*, *Ionaspis*, and *Hymenelia*. To attain these objectives, an anatomical and morphological study was coupled with an investigation using enzyme electrophoresis. Distinct homogeneous groups are delimited using statistical methods, phylogenetic relationships are estimated using cladistics, and relevant classification and nomenclature are reviewed.

Materials and Methods

Herbarium and Field Work. Herbarium specimens were examined at CANL and borrowed from the following herbaria: ASU, COLO, DUKE, H, L, LAM, M, MICH, MIN, NY, NYS, PH, QEF, QFA, TRTC, UC, UPS, US, WIS, and Claire Smith's personal herbarium.

Field work was conducted by the first author in the Mont Albert (2 populations) and St-Jean-Port-Joli (1 population) regions in the province of Québec, in two localities in the Ottawa region of Ontario, and in the following Arctic localities in the Northwest Territories: Iqaluit, Nettilling Lake, and Amadjuak Lake on southern Baffin Island; southern Cornwallis Island; Coppermine; and Cambridge Bay and the Holman region on Victoria Island. In all, 154 populations were sampled. For each population, at least three samples were collected. For 76 populations, five additional specimens were collected for enzyme electrophoretic studies. All specimens collected were deposited in CANL.

Anatomical and Morphological Study. All microscopic observations and measurements were made using a Leitz Dialux 20 EB compound microscope equipped with fluotar and phase contrast objectives and with a Wild-Leitz M-5 dissecting microscope. Both microscopes were equipped with a light blue, "daylight" filter. For color determination of apothecial pigments, hand sections were mounted in distilled water on microscope slides. The pigments in apothecial tissues were also characterized by their reaction to concentrated HNO_3 and 10% KOH applied directly to dry, hand sections.

Apothecial density was determined using a 2.5 × 2.5 mm microquadrat and a dissecting microscope. The quadrat was placed in areas of the thallus where the apothecia were most abundant. Six observations were made whenever possible. Apothecial density was expressed in the keys and descriptions as numbers of apothecia per 6.25 mm^2.

The identification of *Trentepohlia* was based on presence/absence of an orange pigment caused by the presence of cytoplasmic lipid droplets containing carotenoids (Bold and Wynne 1985). For old material, where the orange pigment was degraded, the distinction be-

tween *Trentepohlia* and trebouxioid algae was made using polarized filters to detect the cell wall refringency of *Trentepohlia*. For endolithic thalli, small calcareous rock fragments containing part of the thallus had to be dissolved in 20% HCl solution to reveal algal cells.

Length and width were recorded for a minimum of five ascospores per specimen from freehand sections mounted in distilled water. If considerable variation was observed, measurements were taken from a maximum of 15 ascospores. The presence of a halo (diffuse epispore of ascospores) was determined by introducing India ink into the distilled water under the cover slip.

All apothecial anatomical observations and measurements were made on apothecia sectioned at 12 μm with a freezing microtome and semi-permanently mounted in lactophenol-cotton blue solution (Duncan and James 1970). For uniformity, only radial longitudinal sections (i.e., the largest sections) of apothecia were used. Hyphal tissues of apothecia were described using Korf's (1958) terminology.

To study the apical structure of the ascus, the hymenium was separated from the margins of the apothecia and, if possible, from the thalline tissues under the apothecia. The central part of the apothecia was mounted directly in 1.5% IKI (Lugol's) solution and gently squashed with a dissecting needle using successive rotations to maximize the spreading (Hafellner 1984).

Enzyme Electrophoretic Study. Fresh material was air-dried no more than three days after collection and frozen (−20° C) within approximately two weeks. The thalli were carefully scraped off the rocks under a dissecting microscope to avoid contamination. For some samples, material from different thalli on the same rock or nearby rocks had to be combined to obtain enough material to detect enzyme activity.

The amount of lichen material used for electrophoresis could not be determined precisely because the samples contained rock crystals intermingled with the lichen. Approximately 1 ml of lichen tissue was ground for 15 seconds in liquid nitrogen with a mortar and pestle. Sterile sand, polyvinyl-polypyrrolidone (PVPP), and approximately 8 drops of cold 0.06 M phosphate buffer (pH 6.6) containing 1 mM mercaptoethanol were added to the lichen powder and ground for 1 min. The slurry was transferred to a cold glass tube and homogenized for 1 min with a power-driven Teflon pestle. The slurry was centrifuged and wicks were inserted into the supernatant.

The activity of 18 enzymes was tested (Lutzoni 1990) in each of four gel systems, including a Tris-citric acid system at pH 8.8 and one at pH 8.3 (Gottlieb 1981), a histidine-citric acid system at pH 6.5 (Warwick et al. 1984), and a histidine system at pH 5.7 (Warwick and Gottlieb 1985). Only three enzymes could be scored reliably: a Tris-citric acid system at pH 8.3 was used for phosphoglucoisomerase (PGI), and a histidine system at pH 5.7 was used for isocitrate dehydrogenase (IDH) and 6-phosphogluconate dehydrogenase (6-PGD). A total of 47 populations was studied: 18 populations from the *haematina* group, 20 from the *epulotica* group, one from the *alba* group (a new species similar to *Hymenelia lacustris;* Lutzoni 1994), two from the *lacustris* group, one from *Aspicilia*, one from the *melanocarpa* group, three from *Eiglera*, and one from the *odora* group (Appendix A in Lutzoni 1990, available from the author). Enzyme extractions of free-living *Trentepohlia* found adjacent to *Ionaspis* with different stages of lichenization (where partial integration of *Trentepohlia* in the lichen thallus of *Ionaspis* could be seen), were run simultaneously on the gels with enzyme extracts of the lichen thallus in order to detect enzyme activity of the photobiont, which was then excluded from the cladistic analysis. Since we did not have any algal isolates from lichen species having a trebouxioid photobiont we could not exclude bands specific to these trebouxioid algae. This would not influence the present results since enzyme activity of *Trentepohlia* was very rarely detected in the lichen extracts. We expect the same is true for the trebouxioid algae.

Statistical Procedures. The first step in this study was to identify distinct homogeneous groups within the *Ionaspis-Hymenelia* complex by subjecting morphological and anatomical characters to statistical analyses. Summary statistics were obtained for anatomical-morphological data using the S199 program (Agriculture Canada, Ottawa). The data were then scaled before subjecting them to any subsequent numerical analyses. Since the matrix was a mixture of discrete and continuous characters, Gower's (1971) similarity coefficient was used to measure pairwise similarities. Cluster analysis using the

flexible sorting algorithm (Lance and Williams 1967) with $\alpha = 0.625$ and $\beta = -0.25$, was done using Agriculture Canada's CLUSTRIT (S075) program (Lefkovitch 1981). To execute principal component analysis (PCA) and canonical variate analysis (CVA), PRINCOMP, STEPDISC, DISCRIM and CANDISC programs (SAS Institute Inc. 1985) were used. Equally weighted characters were used throughout this study.

Choice of Specimens and Morphological Characters. In an initial survey of 20 individuals, specimens were selected to represent the range of variation of characters within hymenelioid lichens. All characters used in previous studies on this complex (Magnusson 1933; Eigler 1969; Poelt and Vězda 1981; Hafellner 1984), as well as any additional characters that had proven to be useful in other genera (Poelt 1973; Brodo 1984; Hale 1984; Bellemère and Letrouit-Galinou 1987; Kärnefelt and Mattson 1987), were scored for these 20 individuals, for a total of 92 characters. Thirty-one characters were eliminated after this first step for one of the following reasons: 1) absence of the character in one or more of the 20 specimens; 2) no variation in the data, or 3) the impossibility of reliably describing or measuring a structure due to difficulties with its examination or to excessive variation within the same individual.

Scoring of the remaining 61 characters was then extended to 94 specimens. A cluster analysis using the flexible sorting algorithm was performed on this first data matrix. Only 35 characters (Appendix 1) important for explaining the clusters revealed by PCA and CVA were retained for subsequent numerical analyses. These characters were then scored from 102 additional specimens and the resulting matrix was subjected to the same statistics that were applied to the matrix of 94 specimens. The generic descriptions were based on observations and measurements recorded on approximately 300 specimens (see Lutzoni 1990 for voucher information and the distribution of these specimens among the different taxa surveyed in this study).

Continuous to Discrete Character Conversion for Cladistic Analysis. *Ionaspis*, *Hymenelia*, and *Eiglera* are very cryptic lichens with "simple" morphologies, which limit the potential to find obvious discrete characters. Preliminary cladistic analysis with the 14 discrete characters (Table 1) alone could not resolve relationships clearly and thus continuous characters were included. The statistical procedure leading to the conversion of continuous characters to a discrete form was done using SYSTAT (version 5.2, 1992). First, an analysis of variance (ANOVA) was performed on each character. If the null hypothesis (H_0 = mean of each group is equal) was rejected for a given character, a pairwise mean comparison using the Tukey HSD post hoc test was done. The post hoc test was used since the probability of finding one significant difference by chance alone increases rapidly with the number of pairwise comparisons. The matrix of pairwise comparison probabilities was then used to determine the character state for each group. First, only the probabilities ≤ 0.01 were used to allocate a character state to each group. Then, all the remaining probabilities in the matrix were used, starting with the highest probabilities, to verify the character state allocation. When a conflicting coding was found for two groups, the group with the most intermediate pairwise probabilities (i.e., with probabilities ≤ 0.80 and ≥ 0.01) was given two character states and coded as uncertain states as described by Maddison and Maddison (1992). The results of this procedure are shown in Fig. 1.

Cladistic Analyses. A total of 26 morphological characters was used for the cladistic analysis (Table 1). The three ecological and phytogeographical characters were excluded (Appendix 1). Two other characters (paraphysal ramifications and constrictions) were excluded because they were uninformative. The continuous characters were not subdivided into minimum, maximum, and average values as in the statistical analyses. Three characters were added: pycnidia diameter, conidia length, and conidia width.

For each of the eight distinct homogeneous groups circumscribed by the statistical procedures described above, the morphological data were pooled within each group. These morphologically homogeneous groups also formed the basis for pooling the enzyme electrophoretic data, which were used here as an additional data set to estimate phylogenetic relationships among the homogeneous groups. Since the thallus is haploid, a given allele was scored as absent for a given species group (Table 5) only if it was never detected among all specimens included in the enzyme electrophoretic study for this species group (Appendix A in

TABLE 1. Annotated list of the 26 morphological characters used in the cladistic analysis. Character state distributions in *Eiglera*, *Aspicilia*, and the homogeneous groups delimited within the *Ionaspis–Hymenelia* complex are summarized in Table 4. Numbers in parentheses refer to colors using the U.S. National Bureau of Standards, *Inter-Society Color Council (ISCC) Dictionary of Color Names* (Kelly and Judd 1976) using a chart of centroid colors (Kelly 1965).

Characters	States
1) Apothecial disk color	0 = black (267); 1 = brownish gray (60, 61, 62, 63, 64, 65); 2 = grayish reddish brown (45, 46, 47); 3 = dark brown (55, 56, 57, 58, 59); 4 = dark gray (264, 266); 5 = grayish yellowish brown (75, 80); 6 = pale pink (4, 5, 7, 8, 9, 28, 31, 33); 7 = pale orange yellow (70, 73, 92, 93); 8 = light orange (51, 52, 53); 9 = hyaline (263)
2) Thallus type	0 = endolithic; 1 = epilithic
3) Apothecial margin shape	0 = not prominent; 1 = slightly prominent; 2 = prominent; 3 = very prominent and constricted
4) Photobiont	0 = trebouxioid; 1 = *Trentepohlia*
5) Epihymenial color	0 = blackish green (146, 147, 152, 157); 1 = dark reddish brown (44); 2 = bluish green (160, 161, 164, 165); 3 = black (267); 4 = dark yellowish brown (74, 76, 78); 5 = dark olive brown (95, 96, 111, 114); 6 = bluish black (193); 7 = hyaline (263, 264); 8 = deep orange yellow (68, 69, 82, 88); 9 = strong brown (55, 59)
6) Dark excipulum proprium	0 = absent; 1 = continuous below the subhymenium; 2 = present in the apothecial margin only
7) Ascospore halo	0 = absent; 1 = present
8) Tholus reaction to 1.5% IKI (Lugol's) solution	0 = negative; 1 = positive
9) Paraphyses shape	0 = larger at the apex; 1 = uniform in width
10) Ascospores organization in ascus	0 = uniseriate; 1 = aseriate
11) Hymenial reaction to 1.5% IKI (Lugol's) solution	0 = negative; 1 = positive
12) Hymenial reaction to HNO_3	0 = negative; 1 = positive, violaceous pink; 2 = positive, orange yellow
13) Hymenial reaction to KOH	0 = negative; 1 = positive, dark violet
14) Epipsamma	0 = absent; 1 = present
15) Apothecia density	0 = diffuse; 1 = dense (see Fig. 1)
16) Apothecial disk diameter	0 = small; 1 = medium; 2 = large (see Fig. 1)
17) Apothecial margin thickness	0 = small; 1 = medium; 2 = large (see Fig. 1)
18) Ascospore length	0 = very short; 1 = short; 2 = long; 3 = very long (see Fig. 1)
19) Ascospore width	0 = very narrow; 1 = narrow; 2 = wide; 3 = very wide (see Fig. 1)
20) Hymenium thickness	0 = thin; 1 = thick (see Fig. 1)
21) Subhymenium thickness	0 = thin; 1 = thick (see Fig. 1)
22) Hypothecium thickness	0 = thin; 1 = thick (see Fig. 1)
23) Lateral excipulum proprium thickness	0 = thin; 1 = thick (see Fig. 1)
24) Pycnidium diameter	0 = small; 1 = large (see Fig. 1)
25) Conidium length	0 = short; 1 = long (see Fig. 1)
26) Conidium width	0 = narrow; 1 = wide (see Fig. 1)

Lutzoni 1990). The phylogenetic relationships among the six groups delimited within the *Ionaspis-Hymenelia* complex and the *Eiglera* group were estimated using PAUP version 3.1.1 (Swofford 1993). The most parsimonious trees were found through exhaustive searches. Both morphological and allozyme data sets were analyzed as unrooted networks, and rooted with *Aspicilia* using the Lundberg rooting method (Lundberg 1972). The resulting trees were evaluated by 1,000 bootstrap replications (Felsen-

TABLE 2. List of characters, in decreasing order of importance, best explaining the distribution in multidimensional space of *Eiglera*, *Aspicilia*, and six species groups within the *Ionaspis–Hymenelia* complex. The "x" indicates, for each character, which statistical analysis revealed its importance in explaining the variation. For PCA, the axes (components) were specified.

Characters	PCA axis 1	PCA axis 2	Stepwise discriminant analysis (prob. > *F*)
Ascospore length	x		x (0.0001)
Ascospore width	x		x (0.0001)
Apothecial margin thickness		x	x (0.0001)
Hymenium thickness	x		x (0.0001)
Lateral excipulum proprium thickness		x	x (0.0074)
Apothecial disk diameter			x (0.0001)
Apothecia density			x (0.0001)
Subhymenium thickness			x (0.0011)
Hymenial reaction to HNO_3		x	
Thallus type (epi- or endolithic)		x	
Epihymenial color		x	
Tholus reaction to 1.5% IKI (Lugol's) solution		x	
Substrate reaction to HCl	x		

TABLE 3. Taxa included in the six species groups of the *Ionaspis–Hymenelia* complex. The nomenclature used is prior to this study. See generic descriptions for authorities.

Species group	Taxa
haematina group	*Ionaspis aigneri* (?), *Hymenelia coerulea* (?), *I. cyanocarpa*, *I. fuegensis*, *I. haematina*, *I. heteromorpha*
epulotica group	*Ionaspis arctica*, *I. carnosula*, *Hymenelia epulotica* (= *H. prevostii*), *I. rhodopis*, *H. similis*
melanocarpa group	*Ionaspis melanocarpa*
lacustris group	*Hymenelia lacustris*
alba group	*Ionaspis alba*
odora group	*Ionaspis alpina*, *I. lavata*, *I. odora*, *I.* sp. # 1 (Lutzoni 1990), *I. ventosa*

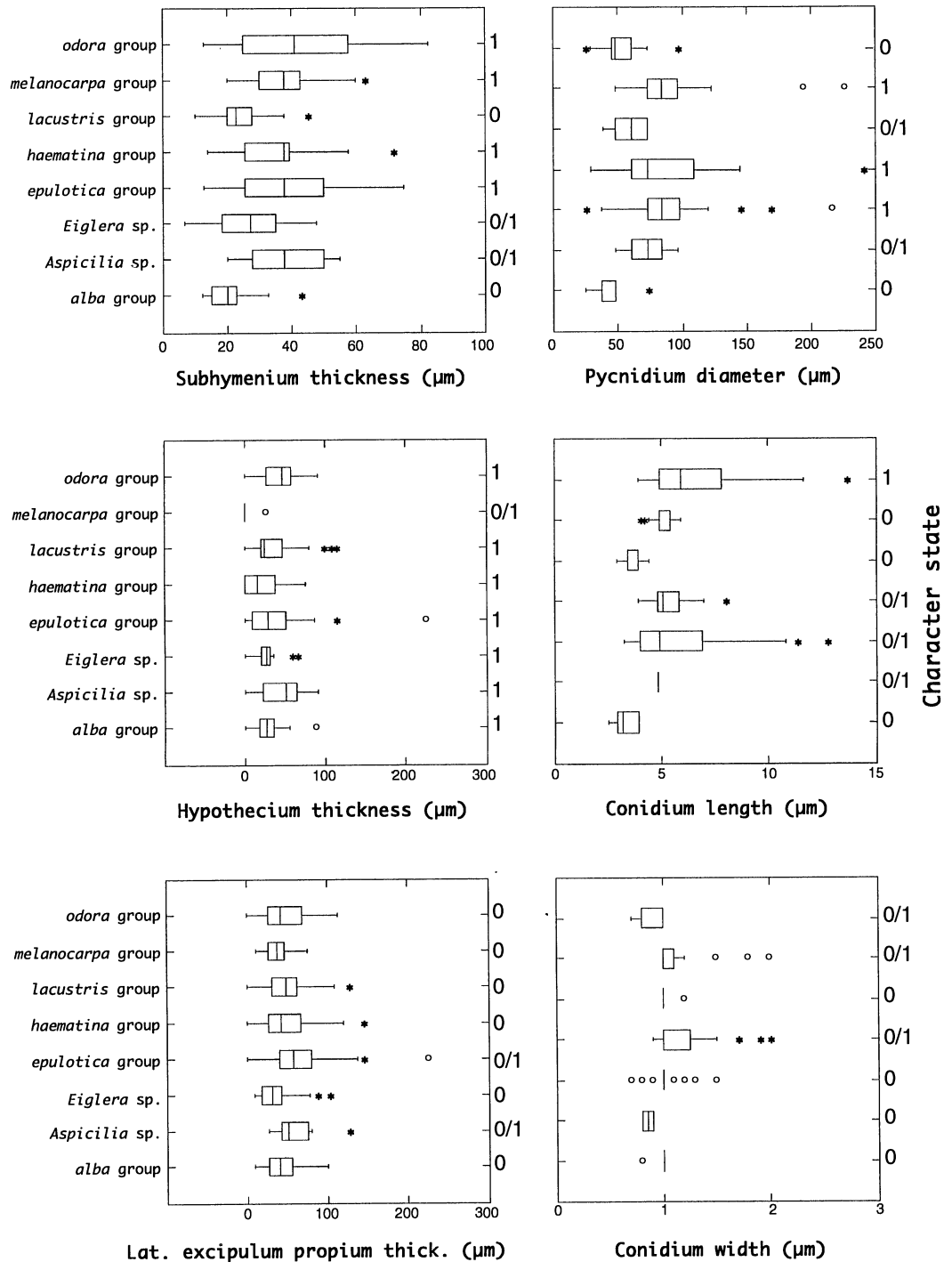
stein 1985) and by determining the decay value (Mishler et al. 1991). The characters were mapped on the topology using MacClade, version 3, with ACCTRAN optimization (Maddison and Maddison 1992).

To determine whether the data sets based on morphology and enzymatic profiles shared a common phylogenetic history and, therefore, could be combined, the protocol by Rodrigo et al. (1993) was applied as described in Lutzoni and Vilgalys (1995). The combined equally weighted analysis was performed on the eight homogeneous groups following the same specifications mentioned above. The phylogenetic network of eight OTU's was rooted using *Aspicilia* as the outgroup (not Lundberg rooted). The same combined phylogenetic analysis was implemented on the six species groups of the *Ionaspis-Hymenelia* complex alone. The phylogenetic network resulting from this analysis on six OTU's was left unrooted.

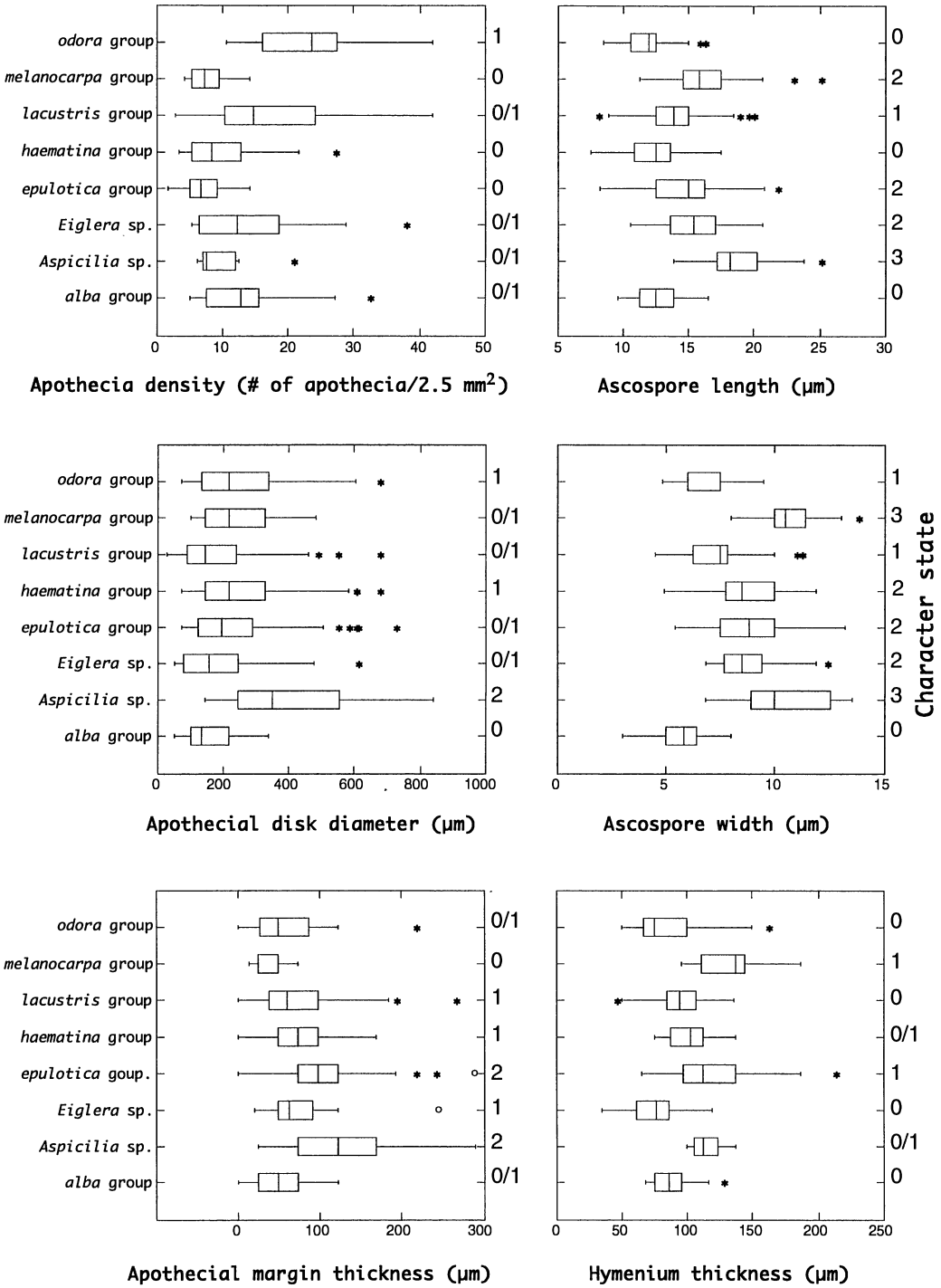
Descriptions. Terminology used to describe the internal anatomy of the apothecia is illustrated in Fig. 2. Measurements are usually given by five numbers [e.g., (1.5–)**4.0**-**8.5**-

→

FIG. 1A, B. Range of variation of continuous characters within homogeneous groups and the allocation of discrete character states. The box plots were produced using SYSTAT (version 5.2, 1992; see SYSTAT Graphics p. 183 for interpretation of box plots beyond the following). The median is marked by the vertical line within the box. The lower and upper hinges form the edges of the central box. The median splits the ordered batch of numbers in half, and the hinges split the remaining halves in half again. The lower and upper inner fences are the lower or upper hinge minus or plus 1.5 Hspread, respectively, where Hspread is comparable to the inter quartile range or midrange. The lower and upper outer fences are the lower or upper hinge minus or plus 3 Hspread, respectively. Values outside the inner fences are plotted with asterisks. Values outside the outer fences are plotted with empty circles. For each continuous character where the null hypothesis (H_0 = mean of each group is equal) was rejected using an ANOVA, a pairwise mean comparison using Tukey HSD post hoc test was done. The matrix of pairwise comparison probabilities was then used to determine the discrete character state for each group. First, only the probabilities ≤ 0.01 were used to allocate a character state to each group. Then, all remaining probabilities in the matrix were used, starting with the highest probabilities, to verify the character state allocation. When a conflicting coding was found for two groups, the group with the most intermediate pairwise probabilities (i.e., with probabilities ≤ 0.80 and ≥ 0.01) was given 2 character states and treated as uncertain states (for example, 0/1) as described by Maddison and Maddison (1992).


odora group
melanocarpa group
lacustris group
haematina group
epulotica group
Eiglera sp.
Aspicilia sp.
alba group
Subhymenium thickness (µm)
Pycnidium diameter (µm)
Hypothecium thickness (µm)
Conidium length (µm)
Lat. excipulum propium thick. (µm)
Conidium width (µm)
Character state

A


odora group
melanocarpa group
lacustris group
haematina group
epulotica group
Eiglera sp.
Aspicilia sp.
alba group
1
0
0/1
0
0
0/1
0/1
0/1
0 10 20 30 40 50
Apothecia density (# of apothecia/2.5 mm2)
0
2
1
0
2
2
3
0
5 10 15 20 25 30
Ascospore length (µm)
1
0/1
0/1
1
0/1
0/1
2
0
0 200 400 600 800 1000
Apothecial disk diameter (µm)
1
3
1
2
2
2
3
0
0 5 10 15
Ascospore width (µm)
Character state
0/1
0
1
1
2
1
2
0/1
0 100 200 300
Apothecial margin thickness (µm)
0
1
0
0/1
1
0
0/1
0
0 50 100 150 200 250
Hymenium thickness (µm)

B

TABLE 4. Matrix of morphological characters used for cladistic analyses of *Eiglera*, *Aspicilia*, and species groups within the *Ionaspis–Hymenelia* complex. The "&" symbol indicates a polymorphism, and the "/" symbol indicates that the state in the taxon is partially uncertain for a given character. Question marks indicate missing data. See Table 1 for the character number and character state descriptions.

Species group	Characters						
	1	2	3	4	5	6	7
haematina group	0	0&1	0&1&2&3	1	0&2&5	1&2	0&1
epulotica group	4&6&7	0&1	0&1&2&3	1	7	0	0&1
melanocarpa group	0	0	1	1	0&2	1	0&1
odora group	1&2&3&4&5	1	0&1&2	1	4&5&7	0&1	0
lacustris group	1&2&3&4&5&6&7&8	0&1	0&1&2&3	0&1	4&5&7&8&9	0	0&1
alba group	4&5&7&8&9	1	0&1&2	0	4&7	0	0&1
Eiglera	0	0&1	1&2&3	0&1	0&1&2&3&4&5&6	2	0&1
Aspicilia	0&1	1	1&2&3	0&1	5	2	0

13.5(–27.0)]. The numbers in parentheses are the most extreme measures recorded. The numbers in bold, but not underlined, are the lower and upper limit of the standard deviation applied to the average. The number in bold and underlined is the overall average. Colors of the disk, thallus and apothecial pigment were reported using the U.S. National Bureau of Standards, *Inter-Society Color Council (ISCC) Dictionary of Color Names* (Kelly and Judd 1976) using a chart of centroid colors (Kelly 1965). The numerical code for each color is included in parentheses.

RESULTS

Delimitation of Distinct Homogeneous Groups within the* Ionaspis-Hymenelia *Complex. The goal of this study was to work at the generic level; therefore, the first step was to break up the dendrogram of 196 specimens (Fig. 3) into the smallest, but statistically significant, partitions possible. Phylogenetic relationships of those homogeneous entities could then be estimated using cladistics. The highest number of distinct clusters found to be statistically significant using CVA was eight (Fig. 3). The generalized distances among the clusters were shown to be highly significant (0.0001 level) for all pairwise distances except between the *alba* and *lacustris* groups, which was 0.0024. The composition of these eight clusters was each dominated by individuals with unique and obvious macroscopic character states. Using these criteria, misclassifications within clusters were identified, corresponding to the letters on the left of the dendrogram of Fig. 3. The terms "species groups" or "homogeneous groups" are henceforth used to designate these eight reclassified clusters as represented by the letters on Fig. 3. No taxonomic rank was given to these species groups, but we are confident that they include one or a few closely related species based on diagnostic morphological characters. Eight characters were shown to be statistically significant by a stepwise discriminant analysis to explain the eight species groups (Table 2). PCA was used to find additional characters associated with these homogeneous groups.

The first two components of the PCA (Fig. 4) explained 30.6% of the total variation, the third 7.3%, while each of the 32 other components explained less than 5.8% of the variation. The species groups revealed by the flexible sorting method occupied more or less distinct regions of the PCA bidimensional projection, except for the *lacustris* group, represented here mostly by the very polymorphic species *Hymenelia lacustris*. The characters underlying this pattern on PCA axes 1 and 2 are listed in Table 2. All species groups with black apothecial disks (i.e., *Aspicilia*, *Eiglera*, *haematina* and *melanocarpa* groups; apothecial disk corresponds to the characters "hymenial reaction to HNO_3" and "epihymenial color" in Table 2) were found in the lower half of the projection compared to species groups having pale apothecia (Fig. 4). Other characters, such as thickness of the apothecial margin, lateral excipulum proprium thickness, thallus type, and tholus reaction to Lugol's solution, contributed to this pattern (Table 2). Ascospore length and width, hymenium thickness, and substrate reaction to HCl were responsible for most of the variation along the first PCA axis.

TABLE 4. Extended.

Characters																		
8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
0	0&1	0&1	0&1	1	0	0	0	1	1	0	2	0/1	1	1	0	1	0/1	0/1
0	0&1	0&1	0&1	0	0	0	0	0/1	2	2	2	1	1	1	0/1	1	0/1	0
0	0&1	0&1	0&1	1	0	0	0	0/1	0	2	3	1	1	0/1	0	1	0	0/1
0	0&1	0&1	0	0&2	1	0	1	1	0/1	0	1	0	1	1	0	0	1	0/1
0	0&1	0&1	0&1	0	0	0&1	0/1	0/1	1	1	1	0	0	1	0	0/1	0	0
0	0	0&1	0	0	0	0&1	0/1	0	0/1	0	0	0	0	1	0	0	0	0
1	0&1	1	0&1	1	0	0	0/1	0/1	1	2	2	0	0/1	1	0	0/1	0/1	0
0	0&1	1	0&1	0	0	0	0/1	2	2	3	3	0/1	0/1	1	0/1	?	?	?

Ascospore length and width, apothecial margin thickness, hymenium thickness, and lateral excipulum proprium thickness were shown to be discriminant characters in both the PCA and the stepwise discriminant analysis in distinguishing these eight species groups.

Subsequent observations showed that some species groups were characterized by the presence or absence of an epipsamma, the presence or absence of halonate ascospores, and phytogeography. In Table 3, species of *Ionaspis* and *Hymenelia* were classified according to the six species groups of the *Ionaspis-Hymenelia* complex (i.e., excluding *Aspicilia* and *Eiglera*) as obtained through this statistical procedure.

***Phylogenetic Relationships among Homogeneous Groups of the* Ionaspis-Hymenelia *Complex and* Eiglera.** ANATOMICAL AND MORPHOLOGICAL DATA. The distribution of morphological character states is summarized in Table 4. Equally weighted parsimony analysis yielded three equally most parsimonious topologies that differ only in the placement of *Eiglera* (Fig. 5). Lundberg rooting resulted in a three-way tie for topological positions where *Aspicilia* could be inserted as an outgroup; these three positions were identical for the three unrooted topologies. When *Eiglera* and *Aspicilia* were excluded from the analysis, only one most parsimonious topology was obtained (Fig. 6).

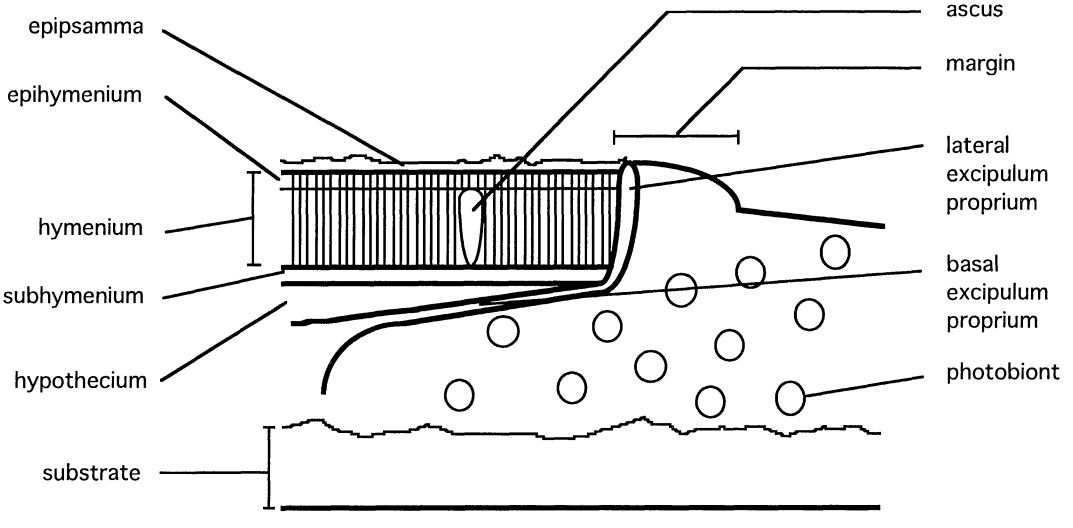


FIG. 2. Schematic representation of a radial longitudinal section of an hymenelioid apothecium.

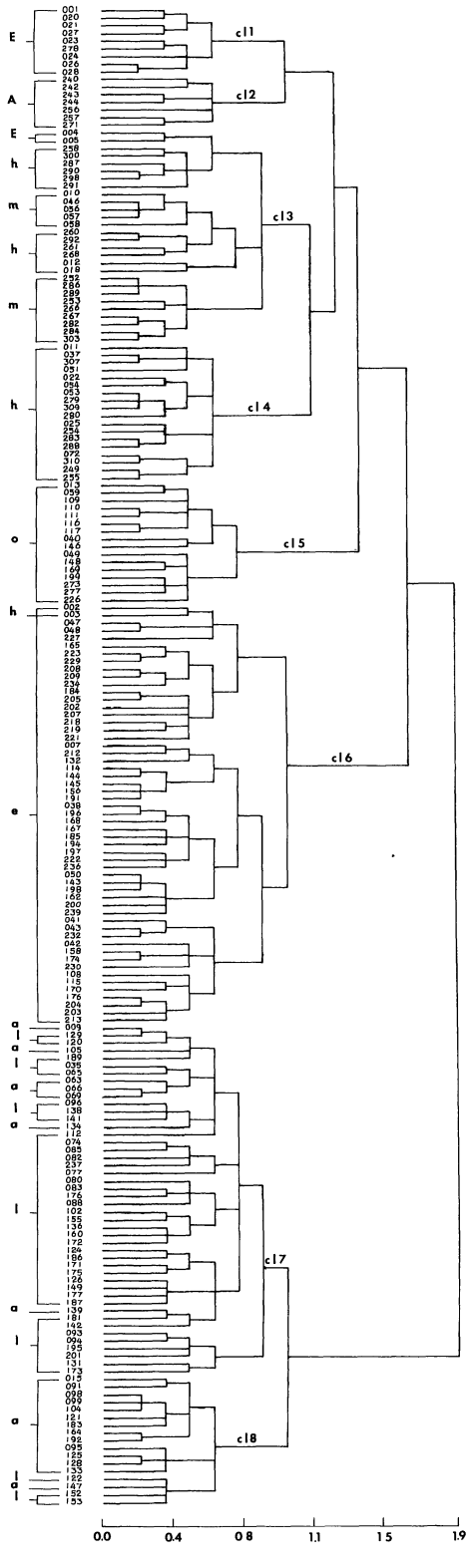
TABLE 5. Matrix of allozyme data for cladistic analysis of *Eiglera*, *Aspicilia*, and species groups within the *Ionaspis–Hymenelia* complex. 0 = allele absent, 1 = allele present at least once.

	Alleles numbered within each isozyme																													
	6-PGD													IDH		PGI														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	1	2	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
haematina group	0	1	1	1	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	1	1	1	1	1	0	1	0	1	1	0	0
epulotica group	0	1	1	0	1	1	1	0	0	0	0	0	0	0	0	1	0	0	0	1	1	1	1	0	0	0	0	0	0	1	1
melanocarpa group	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	1	0	0	1	0	0	0	0	0
odora group	1	0	0	0	0	0	0	0	0	0	0	1	1	1	1	0	1	1	1	1	0	0	0	0	1	0	0	0	0	0	0
lacustris group	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	1	1	1	1	0	1	0	0	0	0	0	0	0	1	0	0
alba group	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0
Eiglera flavida	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	1	0	0	0	1	0	1	1	0	0	1	0	0	0	0	0
Aspicilia cinerea	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0

The internode joining the *epulotica-haematina-melanocarpa* groups to the *alba-odora-lacustris* groups and the internode within the *epulotica-haematina-melanocarpa* network are well supported as shown by the high bootstrap values and decay indices (Fig. 6).

ALLOZYME DATA. Three enzyme systems were resolved for the *Ionaspis-Hymenelia* complex, *Eiglera*, and *Aspicilia*, for which 31 allozymes were recorded (Table 5). Of these, 17 were phylogenetically informative characters when a cladistic analysis was applied to the eight homogeneous groups, and 16 allozymes were phylogenetically informative when the analysis was restricted to the six homogeneous groups forming the *Ionaspis-Hymenelia* complex. Equally weighted parsimony analysis of *Eiglera flavida* (Hepp) Hafellner and the six homogeneous groups within the *Ionaspis-Hymenelia* complex [rooted with *Aspicilia cinerea* (L.) Körb.] revealed one most parsimonious topology of 25 steps (Fig. 7A). The placement of *Eiglera flavida* and *Aspicilia cinerea* was unequivocal. *Eiglera* was found to be within the *melanocarpa-epulotica-haematina* clade. The allozyme data suggest that the network should be rooted at the internode linking two major clades (i.e., between the *alba-odora-lacustris* and the *epulotica-haematina-melanocarpa* clades) rather than within the *epulotica-haematina-melanocarpa* clade, as suggested by the morphological data (Figs. 5 and 7A).

To compare with the unrooted network of the six homogeneous groups of the *Ionaspis-Hymenelia* complex based on morphological data (Fig. 6), a second cladistic analysis using the allozyme data set was restricted to those six groups (i.e., excluding *Aspicilia cinerea* and *Eiglera flavida*). One most parsimonious topology of 20 steps was obtained (Fig. 7B). As in the morphological analysis, the best supported internode in the allozyme analysis was between the *epulotica-haematina-melanocarpa* groups and the *alba-odora-lacustris* groups, with a bootstrap value and decay value of 99% and 5, respectively. Contrary to the topology based on morphology, all the internodes of the topology revealed by the allozyme analysis were very strongly supported (Figs. 6 and 7B). Moreover the resolution within the *epulotica-haematina-melanocarpa* groups and the *alba-odora-lacustris* groups based on allozyme electrophoretic data differs from the resolution obtained with the morphological data. The strongest conflict occurs within the *epulo-*



tica-haematina-melanocarpa groups, where both data sets strongly support a different topology with the same bootstrap value of 92% and decay value of 3 (Figs. 6 and 7B).

HOMOGENEITY TEST AND COMBINED DATA SETS. To decide whether the two data sets should be combined, we applied the protocol by Rodrigo et al. (1993) as implemented in Lutzoni and Vilgalys (1995). This procedure is an attempt to determine whether two different data sets share the same phylogenetic history. For this series of tests the six species groups, *Eiglera*, and *Aspicilia* were analyzed simultaneously, i.e., without excluding *Aspicilia* for Lundberg rooting. This way six unrooted most parsimonious trees were obtained from the morphological data set and one unrooted most parsimonious tree was revealed by the enzyme electrophoretic data set. The pairwise symmetric-difference between the most parsimonious tree based on allozymes and the six most parsimonious unrooted trees based on morphology was equal to 8. The probability of obtaining a symmetric-difference of 8 for eight taxa is 0.0314 (Hendy et al. 1984, Page 1989). Therefore, we can reject the null hypothesis at the 0.05 level that shared components between the allozyme tree and the six morphological trees are due to chance.

The second step in Rodrigo's protocol is to determine the variability associated with the morphological and molecular trees respectively. A total of 12,099 most parsimonious trees was obtained from the 1,000 bootstrapped morphological data sets, whereas the allozyme treefile contained 3,038 most parsimonious trees resulting from this procedure. A total of 795 trees

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FIG. 3. Dendrogram produced by the flexible sorting method and representing the similarity pattern within the *Ionaspis-Hymenelia* complex in relation to the genera *Eiglera* and *Aspicilia*, based on 35 characters (see Appendix 1). The numbers 1 to 8 preceded by "cl" identify the internal branches supporting the eight clusters found to be statistically significant using CVA. The letters (associated with the specimen numbers) correspond to the following homogeneous groups delimited within this study: E = *Eiglera*, A = *Aspicilia*, m = *melanocarpa* group, h = *haematina* group, o = *odora* group, e = *epulotica* group, l = *lacustris* group, and a = *alba* group. The specimen number corresponds to the reference number for each individual in the anatomical and morphological study.

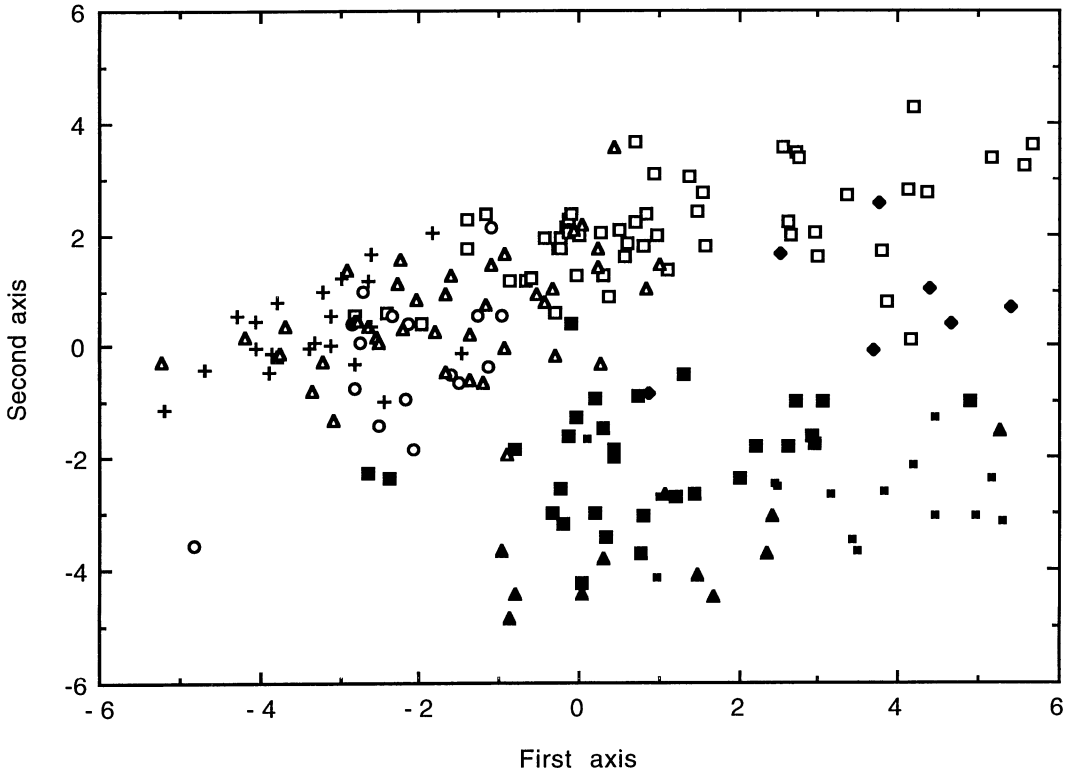


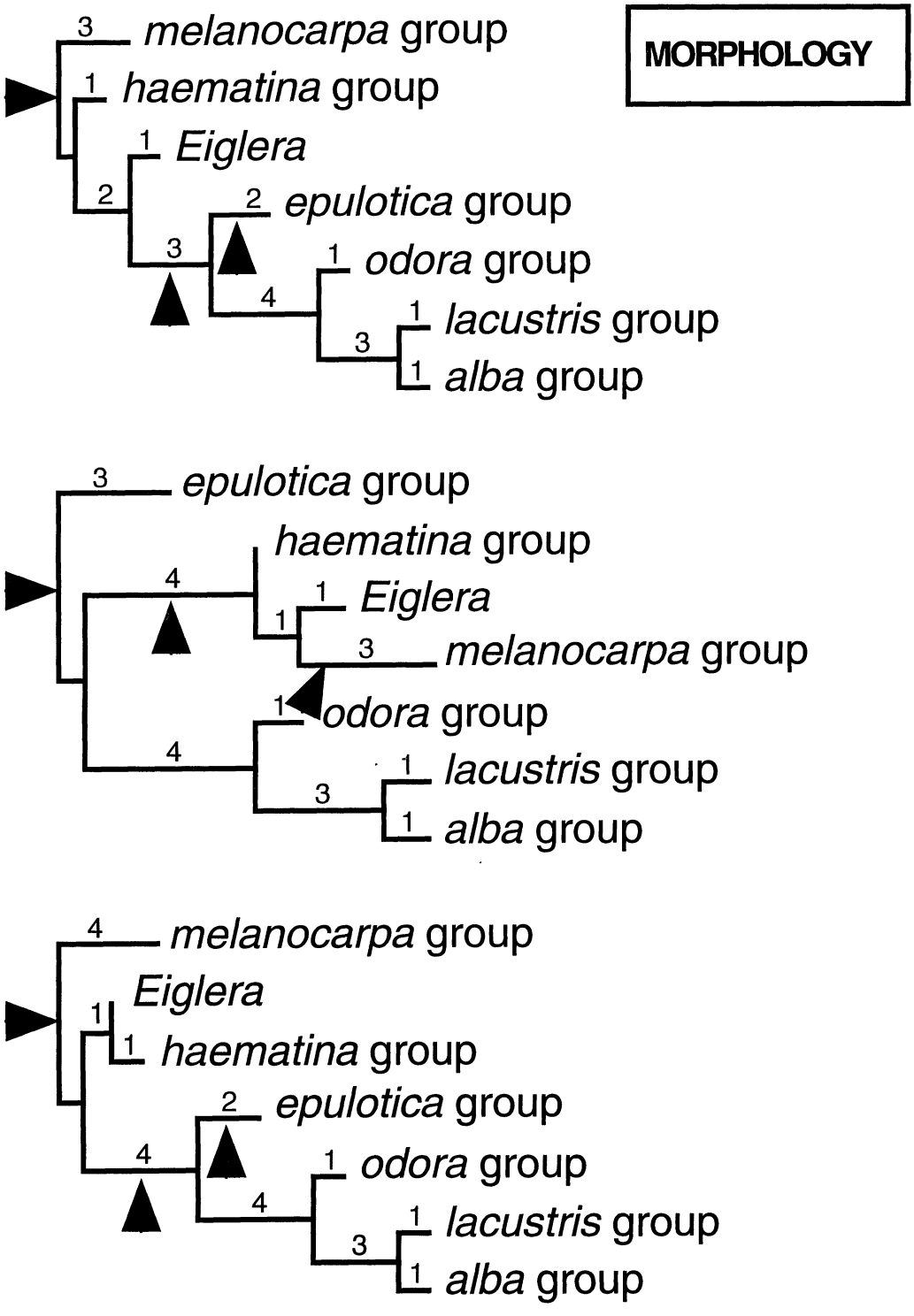
FIG. 4. Projection of 196 specimens from the *Ionaspis-Hymenelia* complex and related genera onto the first two principal component axes using 35 characters (Appendix 1). The symbols indicate the species groups revealed in Fig. 2: ▲ *Eiglera*, ◆ *Aspicilia*, ■ *melanocarpa* group, ■ *haematina* group, □ *epulotica* group, ○ *odora* group, △ *lacustris* group, + *alba* group.

was common to both the morphology and allozyme treefiles. The final step of the protocol developed by Rodrigo et al. (1993) is to test whether the observed symmetric-difference of 8, measured between the morphological and molecular trees, is due to sampling error. If more than 95% of the expected differences in the null distribution are smaller than the observed difference (= 8), then the null hypothesis that the observed distance between the morphological and molecular trees is due to sampling error is rejected. When this test was applied to the morphological data sets 95% of the expected differences in the null distribution were smaller than the observed difference (= 8). Based on the null distribution of differences generated from the allozyme data set, 68% of the expected differences were smaller than the observed difference (= 8). Therefore, we cannot reject the null hypothesis that the observed symmetric-difference of 8 is due to sampling error (Fig. 8). This series of tests by Rodrigo et al. (1993) suggests that the two data sets should be combined (Lutzoni and Vilgalys 1995).

One most parsimonious tree of 58 steps (CI = 0.71, RI = 0.64, RC = 0.45) was revealed by an exhaustive search on combined equally weighted characters (Fig. 9A). This topology is identical to the single most parsimonious tree obtained from the allozyme data (Fig. 7A). A

→

FIG. 5. Three equally most parsimonious topologies (CI = 0.91, RI = 0.85, and RC = 0.77) found in the equally weighted analysis of morphological data recorded on *Eiglera* and homogeneous groups of the *Ionaspis-Hymenelia* complex (Table 4). Branch lengths are proportional to the number of character state changes



indicated above each branch. Arrows correspond to a three-way tie found using Lundberg rooting with *Aspicilia*.

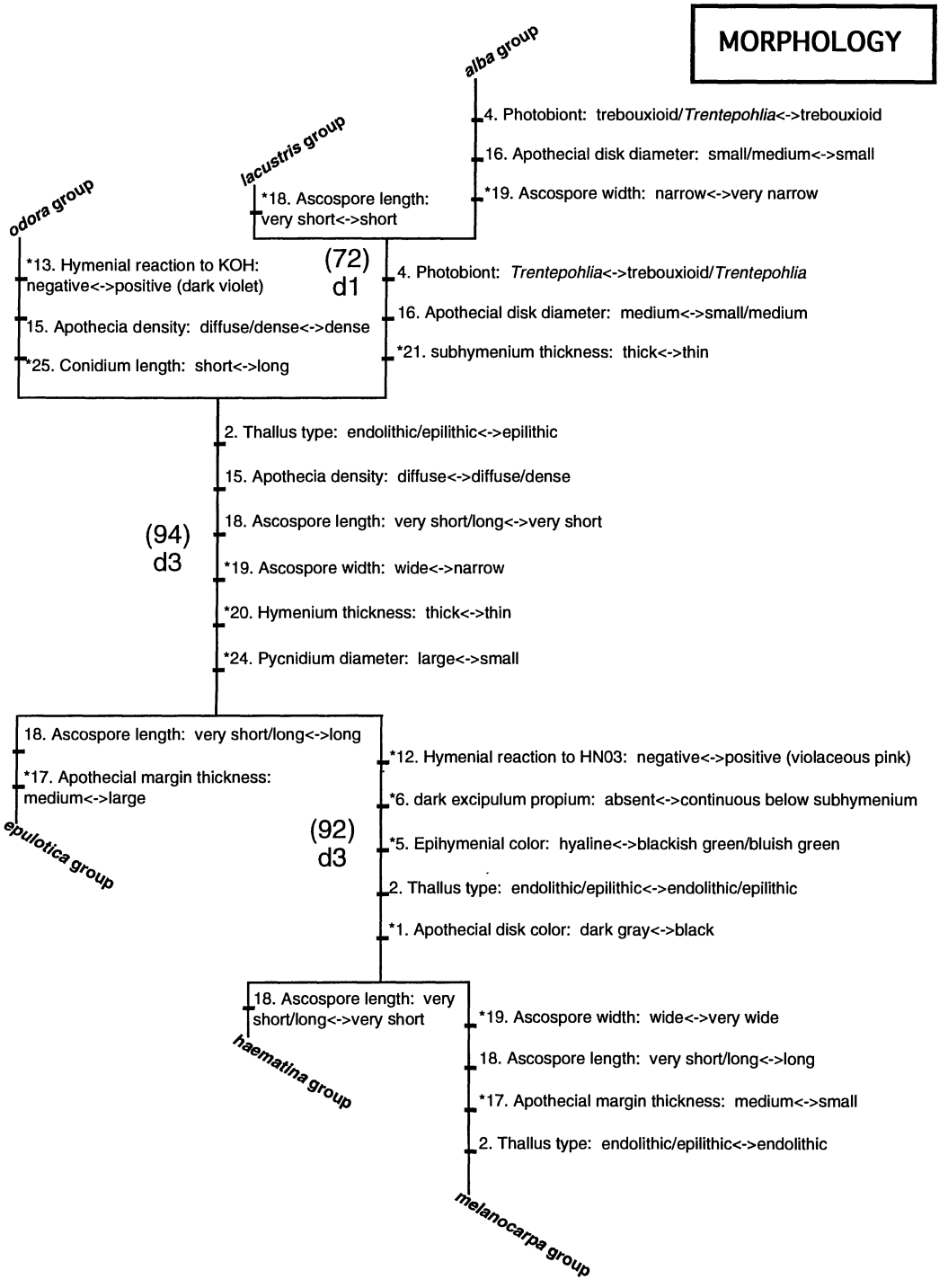


FIG. 6. The most parsimonious unrooted topology (CI = 0.95, RI = 0.89, and RC = 0.84), based on morphology, yielded by equally weighted parsimony analysis when confined to the six homogeneous groups of the *Ionaspis-Hymenelia* complex (i.e., excluding *Aspicilia* and *Eiglera*; Table 4). The state changes preceded by an asterisk are unambiguous. The numbers in parentheses reflect the percentage of 1,000 bootstrap replications that maintained the specified internode. The decay value is preceded by "d."

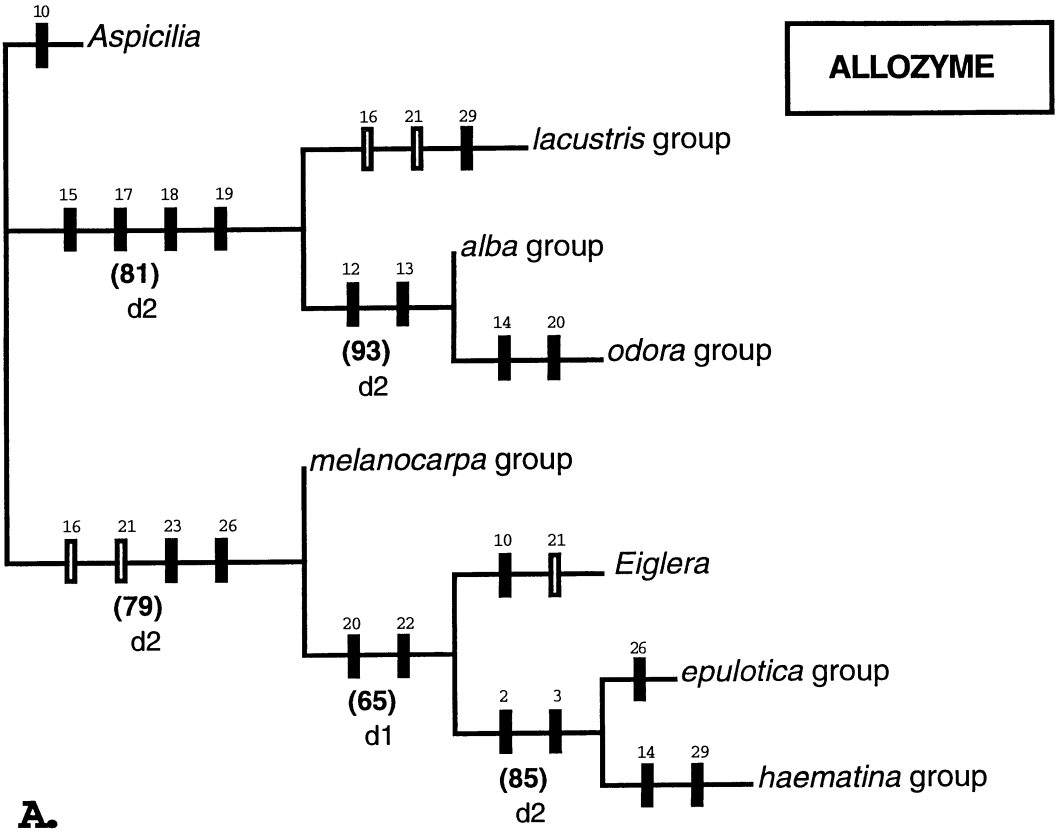
single most parsimonious tree of 44 steps (CI = 0.80, RI = 0.71, RC = 0.56) was generated from the analysis restricted to the six species groups of the *Ionaspis-Hymenelia* complex (Fig. 9B). This topology is different from all most parsimonious trees (Figs. 6 and 7B) obtained from the two data sets when analyzed separately. However, it is one of the two next most parsimonious trees (one step longer) generated by the analysis of the morphological data set, and the single next most parsimonious tree revealed by the analysis of the allozyme data (three steps longer than the most parsimonious tree). The single most parsimonious tree from the combined analysis represents our best estimate for the relationships among the six species groups of the *Ionaspis-Hymenelia* complex (Fig. 9B). It is a composite topology of the most parsimonious topologies based on morphology and allozymes separately. The relationships among the *alba, odora,* and *lacustris* groups are the same as provided by the phylogenetic analysis of allozymes. The relationships among the *epulotica, melanocarpa,* and *haematina* groups revealed by the combined analysis is identical to the analysis of the morphological data. As a result of the conflicting support provided by the two different data sets, especially for the relationships among the *epulotica, melanocarpa,* and *haematina* groups, the resolution within the two main subgroups is less supported in the combined analysis, suggesting that another source of data is needed to confirm this result.

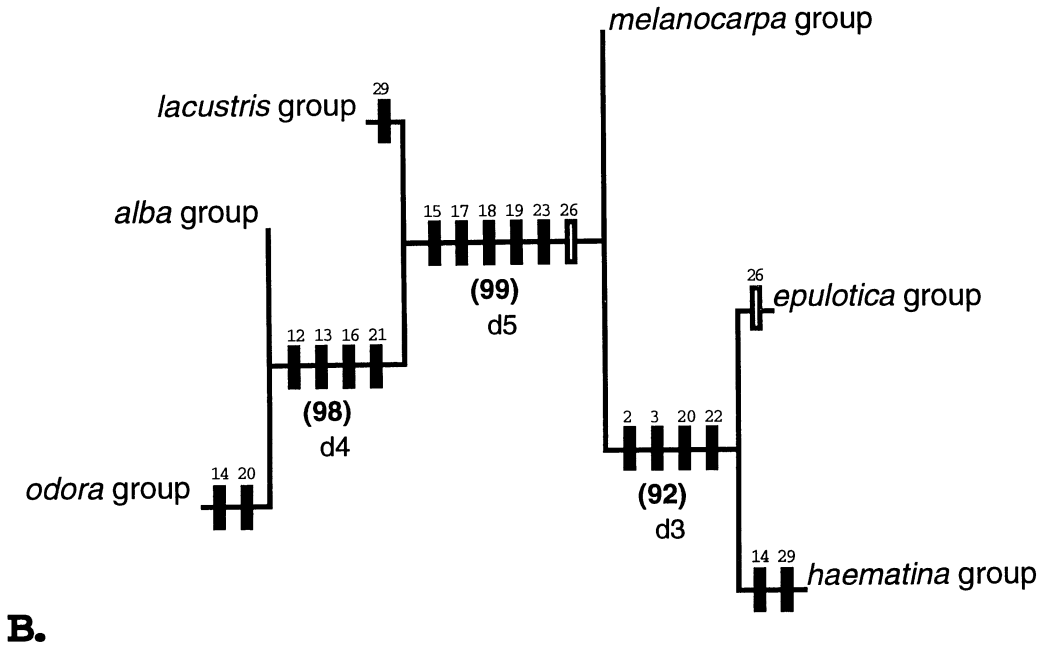
Discussion

Recognition of Genera within the* Ionaspis-Hymenelia *Complex. The critical step in this study was to identify monophyletic generic entities among the six species groups. The separate analysis of allozymes and the combined analysis, using *Aspicilia* as an outgroup (Figs. 7A and 9A), revealed two major clades, the *alba-odora-lacustris* clade and the *epulotica-haematina-melanocarpa* clade. The internode between these two major groups was the only congruent result from the separate and combined analyses of these two data sets, and was consistently well supported by both data sets (Figs. 6, 7B, and 9B). These two clades within the *Ionaspis-Hymenelia* complex are considered here as potential genera. Both groups in the complex have nomenclaturally valid names. The genus *Hymenelia* s. str., with *H. prevostii* (in the *epulotica* group) as its type, now includes the *melanocarpa, haematina* and *epulotica* groups.

The name that should be used for the *alba-odora-lacustris* clade depends on its relationship with the *suaveolens* group, which contains the type species of the genus *Ionaspis.* The *suaveolens* group [*I. fuscoclavata* Eitner, *I. granvina, I. handelii* Zahlbr., and *I. suaveolens* (Fr.) Th. Fr. ex Stein], best characterized by an HNO_3 negative dark green epihymenium, was not included in this study since no specimens were found in North America, precluding any isozyme work on these taxa. Two specimens from the type material of *Ionaspis chrysophana* (Körb.) Th. Fr. ex Stein (≡ *I. suaveolens,* Table 6), described as part of this study, were included as one OTU in a cladistic analysis to determine its phylogenetic relationship within the *Ionaspis-Hymenelia* complex. When the analysis was performed with *Aspicilia* and *Eiglera* it was not possible to determine the phylogenetic relationship of *I. suaveolens* due to a lack of resolution. When *Aspicilia* was removed from the analysis one most parsimonious tree was obtained with *I. suaveolens* nested within *Hymenelia.* However, bootstrap and decay analyses show no support for this topology with bootstrap values ≤ 56% (1,000 replications) and decay values of 1 for each internode. When both *Aspicilia* and *Eiglera* were removed from the analysis three equally most parsimonious trees were obtained all showing *I. suaveolens* well nested within *Ionaspis.* This *Ionaspis* group, including *I. suaveolens,* was better supported with bootstrap values of 64% and decay value of 1, but the support provided by the morphological data is still too weak to formulate any solid hypothesis about the relationship of *I. suaveolens* to the rest of the *Ionaspis-Hymenelia* complex. This means that molecular data, as an independent phylogenetic estimate, will be needed to fully understand the phylogenetic relationships of the *suaveolens* group to the rest of the *Ionaspis-Hymenelia* complex. Until new data, at the species level, are gathered to address this specific question, the *alba-odora-lacustris* clade is considered part of *Ionaspis.*

Acetone-insoluble epihymenial pigments are considered to be efficient generic discriminant characters within the hymenelioid lichenized fungi (Magnusson 1933; B. J. Coppins, unpubl. data). As pointed out by Jørgensen (1989), however, the genetic significance of such characters is not uniform among the different genera. The


ALLOZYME
10
Aspicilia
16 21 29
lacustris group
15 17 18 19
(81)
d2
alba group
12 13
(93)
d2
14 20
odora group
melanocarpa group
16 21 23 26
(79)
d2
10 21
Eiglera
20 22
(65)
d1
26
epulotica group
2 3
(85)
d2
14 29
haematina group
A.


melanocarpa group
lacustris group
29
alba group
15 17 18 19 23 26
(99)
d5
26
epulotica group
12 13 16 21
(98)
d4
2 3 20 22
(92)
d3
odora group
14 20
14 29
haematina group
B.

apothecial disk pigments in *Hymenelia*, as newly circumscribed here (black apothecia in the *haematina* and *melanocarpa* groups, and pinkish apothecia in the *epulotica* group), were sufficiently congruent with other characters to support the *haematina* group joining the *melanocarpa* group (Fig. 6). However, it could be argued that the shared character states supporting the *haematina-melanocarpa* grouping are not independent and the joining of these two homogeneous groups is, therefore, artifactual. Moreover, contrary to the morphological data, the allozyme data suggest that the *epulotica* group should join the *haematina* group (Fig. 7B). Based on results from allozyme data, the apothecial pigment difference between the *epulotica* and *haematina* groups might be the result of recent genetic divergence. An extended molecular study at the species level within *Hymenelia* might also reveal whether disk color is homoplastic among species or even polymorphic within species.

Field observations also support the monophyly of a *epulotica-haematina* group (Fig. 9A). Populations of the *haematina* group were rarely found without representatives of the *epulotica* group nearby. Their thalli can be so similar and intermixed that Lynge (1926) proposed that in *Ionaspis schismatopis* (Nyl.) Hue (synonym of *Hymenalia heteromorpha* (Kremp.) Lutzoni, part of the *haematina* group) the color of the disk changes from pale pinkish to purely black on the same thallus. We concur with Magnusson (1933), however, in seeing no transition between these two types of apothecia in the field, and no thallus that produced both pinkish and black apothecia. Intermixed individuals with black or pinkish apothecia and no obvious delimitation of their respective thalli were frequently seen in the Canadian Arctic. Therefore, it is possible that certain species, now separated into the *haematina* group and the *epulotica* group based on similarity, might be sister species or even conspecific.

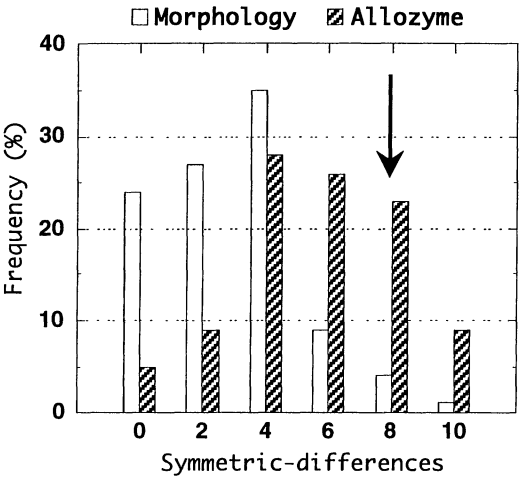


FIG. 8. Null distribution of pairwise symmetric-differences between most parsimonious trees obtained from two sets of 100 bootstrapped datasets, where the trees obtained from the bootstrap analysis in both sets are estimating the same phylogeny. The white and hatched bars represent the null distribution for the morphological and allozyme data sets, respectively. The arrow indicates the observed symmetric-difference between the most parsimonious morphological and allozyme trees.

Both combined and allozyme data sets supported the *alba-odora-lacustris* clade, here recognized as *Ionaspis*. However, the resolution within *Ionaspis* is uncertain due to conflicting relationships suggested by the two independent data sets. The results based on morphology weakly support the joining of the *lacustris* group to the *alba* group (Fig. 6), whereas the allozyme data strongly suggest that the *alba* group and the *odora* group form a monophyletic lineage (Fig. 7A). To fully resolve the relationships within *Ionaspis* as circumscribed in this study, a morphological and molecular investigation needs to be done at the species level and must also include species in the *suaveolens* group. From

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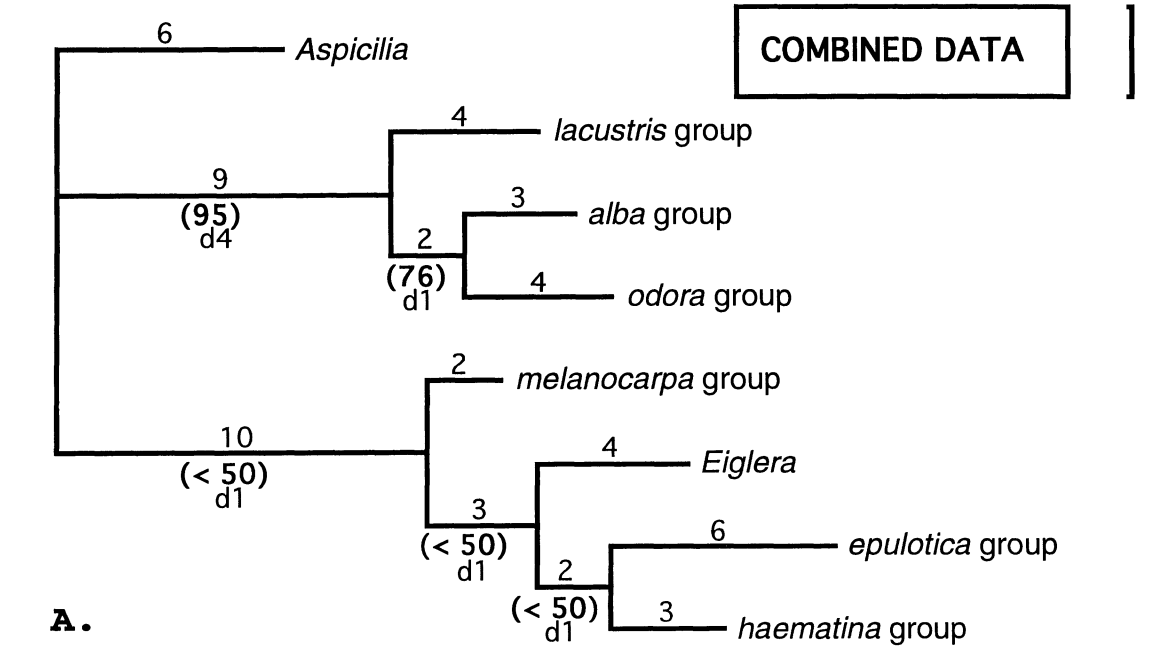
FIG. 7. The single most parsimonious tree obtained from a cladistic analysis of allozyme characters of: A. the six homogeneous groups within the *Ionaspis-Hymenelia* complex and *Eiglera flavida*; the tree shown here is rooted with *Aspicilia cinerea* (25 steps; CI = 0.68, RI = 0.73, RC = 0.50). B. the six homogeneous groups within the *Ionaspis-Hymenelia* complex alone; the tree shown is unrooted (20 steps; CI = 0.80, RI = 0.82, RC = 0.66). Characters and character states are shown in Table 5. Unambiguous changes are represented by black rectangles, ambiguous changes by open rectangles. Numbers in parentheses are the percentage of 1,000 bootstrap replications that supported the specified internode. The decay value is preceded by "d."



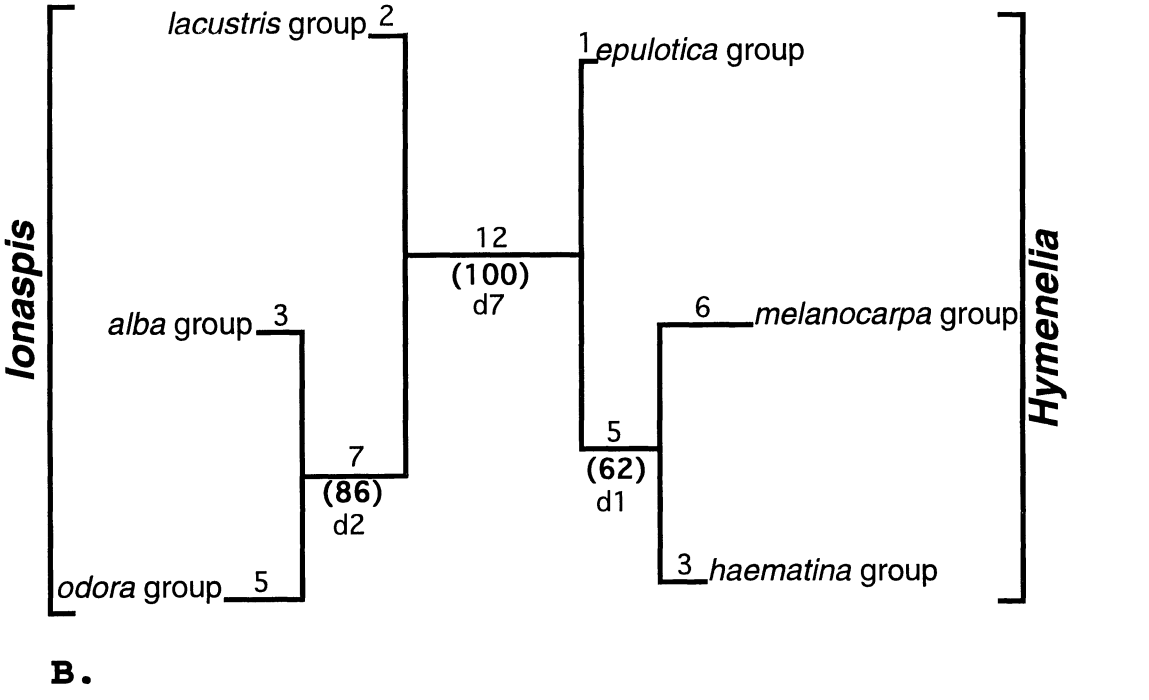


FIG. 9. The single most parsimonious tree obtained from a cladistic analysis of combined data sets of: A. the six homogeneous groups within the *Ionaspis-Hymenelia* complex, *Eiglera flavida*, and the outgroup *Aspicilia*

morphology, it is expected that *Ionaspis* includes three monophyletic groups: 1) the *alba-lacustris* clade; 2) the *odora* clade with *I. alpina* Zahlbr., *I. lavata* H. Magn., *I.* sp. #1 (sensu Lutzoni 1990), *I. odora* (Ach.) Th. Fr. ex Stein, and *I. ventosa* P. M. Jörg. & R. Sant. (all species HNO_3+ orange and KOH+ violet epihymenium), and 3) the *suaveolens* clade with *I. suaveolens* (Fr.) Th. Fr. ex Stein, *I. fuscoclavata* Eitner, *I. granvina* P. M. Jörg. & R. Sant. and *I. handelii* Zahlbr. (all species HNO_3 negative and KOH negative epihymenium). If these phylogenetic relationships receive additional support, what was once a phylogenetic problem will become a ranking problem. Given that these three clades are monophyletic, they might be considered separate genera.

***Comparison of Hymenelioid Genera and* Aspicilia.** The most readily observable characters that differentiate *Aspicilia, Hymenelia,* and *Ionaspis* are listed in Table 6. Since our study did not include *Ionaspis odora* and *I. suaveolens,* two critical European species of the genus *Ionaspis,* Table 6 also includes descriptions made by the first author of the type material for these two species.

The most diagnostic character in the hymenelioid lichens is the color of the apothecial disk and the following related characters: epihymenial color, HNO_3 reaction, and KOH reaction. *Eiglera* is readily distinguishable by its IKI+ tholus. *Hymenelia* has wider ascospores, thicker hymenium, lower apothecial density, and broader pycnidium diameter than *Ionaspis.* Only *Hymenelia* has calcicolous and endolithic species (Table 6). *Aspicilia,* as represented by the *gibbosa* group (Magnusson 1939), is distinct from the hymenelioid lichens in having secondary metabolites including β-orcinol depsidones, larger ascospores, much longer conidia, and a wide geographic distribution.

Status and Phylogenetic Relationships of* Eiglera; *the Importance of Ascus Apical Structure in the Classification of Ascomycetes. A detailed molecular study, as an additional independent phylogenetic estimate, of *Eiglera* in relation to *Hymenelia* is needed before any definitive statement can be made to clarify the status of *Eiglera.* Due to the unresolved position of *Eiglera,* it is preferable at this time to maintain it as a distinct genus. However, it is noteworthy that the morphological and molecular evidence in this study has consistantly shown *Eiglera* to be closely related to *Hymenelia* (Figs. 5, 7A, and 9A). There is only weak support for *Eiglera* as a part of *Hymenelia.* However, these results strongly suggest that *Eiglera* should be classified at least within the same family, the Hymeneliaceae.

Were it not for the unique apical structure of its asci, as revealed by Lugol's solution, *Eiglera* would be classified within *Hymenelia.* Except for this character and its distinctive paraphyses, no other obvious characters distinguish *Eiglera* from *Hymenelia* (Table 6). During field work, the first author noticed that *Eiglera flavida* tolerates stronger water currents than any other aquatic hymenelioid lichen in the Arctic and seemed to be restricted to this type of habitat. Neighboring sympatry would therefore best characterize the distribution of *Eiglera* when compared to *Ionaspis* and *Hymenelia.* The habitat characteristics of *E. homalomorpha* (Nyl.) Clauzade & Cl. Roux, the only other species in the genus, should be determined before this observation can be generalized for the genus as a whole. *Eiglera* and *Hymenelia* are the only genera that have endolithic and epilithic thalli, further evidence of their close relationship (Table 6).

A close phylogenetic relationship of *Eiglera* to *Hymenelia,* indicated by both data sets, raises important questions about the significance given by Hafellner (1984) to the apical structure of asci on which to base generic and family level classifications. Hafellner (1984) segregated *Aspicilia flavida* (Hepp) Rehm from the genus *Aspicilia* to form the genus *Eiglera* and the family Eigleraceae, based mainly on one of the five principles he developed: "as a rule, different types of asci do not occur in the same genus (or family). Thus, the genus (or family) is usually defined by the type of ascus. This signifies that

←

cinerea (58 steps; CI = 0.71, RI = 0.64, RC = 0.45). B. the six homogeneous groups within the *Ionaspis-Hymenelia* complex alone; the tree shown is unrooted (44 steps; CI = 0.80, RI = 0.71, RC = 0.56). Characters and character states are shown in Tables 4 and 5. Branch lengths are proportional to the number of ambiguous and unambiguous character state changes written above each branch. Numbers in parentheses are the percentage of 1,000 bootstrap replications that supported the specified internode. The decay value is preceded by "d."

TABLE 6. Diagnostic comparisons among hymenelioid lichens and the related genus *Aspicilia*.

Diagnostic characters	*Hymenelia*	*Ionaspis odora* Holotype Acharius # 65	*Ionaspis* includes: *odora* group (i.e., *I. lavata* and *I.* sp. #1), *lacustris* and *alba* groups	*Ionaspis chrysophana* type specimens L 8850: 1 and 2	*Eiglera*	*Aspicilia gibbosa* group (sensu Magnusson 1939)
Thallus type	epi- or endolithic	epilithic	epilithic	epilithic	epi- or endolithic	epilithic
Apothecia density (# of apothecia/2.5 mm^2)	(1.7–)**4.1–8.7–13.3**(–27.0)	18	(2.8–)**7.8–16.5–25.2**(–42.2)	(10.0–)**13.7–15.7–17.7**(–20.0)	**5.3–20.0–66.0**	—
Apothecial disk color	black or pinkish	strong brown to strong yellowish brown	whitish or rusty brown to yellowish brown or grayish to almost black	black	black	black
Epipsamma	absent	absent	present or absent	absent	absent	absent
Epihymenial color	Bluish green, olive green or hyaline	hyaline (too pale to be distinguished)	hyaline or yellowish to brownish	very dark green	green to bluish green, also olive brown	olive yellow or olive brown
Hymenial reaction to HNO_3	positive: violaceous pink or negative	positive: orange yellow	positive: orange yellow or negative	negative (= positive color intensified)	positive: violaceous pink	negative (= positive pale yellow-green)
Hymenial reaction to KOH	negative	positive: dark violet	positive: dark violet or negative	negative	negative	negative (= positive deep yellow to olive-brown)
Typical lichen secondary metabolites including β-orcinol depsidones	absent, except for *Porpidia pseudomelinodes*, which was transferred to *Hymenelia* by Gowan and Ahti (1993) as *H. ochrolemma* (Vain.) Gowan & Ahti	—	absent	—	absent	present
Tholus reaction to 1.5% IKI (Lugol's) solution	negative	negative	negative	negative	positive	negative
Paraphysal ramifications	dichotomously ramified at the apex and below the apex	dichotomously ramified at the apex	simple, or dichotomously ramified at the apex, sometimes below the apex	dichotomously ramified at the apex	simple, dichotomously ramified at the apex, rarely lower	simple or ramified at the apex and/or below

TABLE 6. Continued.

Diagnostic characters	*Hymenelia*	*Ionaspis odora* Holotype Acharius # 65	*Ionaspis* includes: *odora* group (i.e., *I. lavata* and *I.* sp. #1), *lacustris* and *alba* groups	*Ionaspis chrysophana* type specimens L 8850: 1 and 2	*Eiglera*	*Aspicilia gibbosa* group (sensu Magnusson 1939)
Paraphysal constrictions	not constricted to moniliform	not constricted	slightly constricted, submoniliform, or moniliform	submoniliform	moniliform or with or without constrictions	sub- or moniliform, or slightly constricted, rarely no constriction
Paraphyses shape 1	larger at apex or uniform in width	uniform in width	generally larger at apex	larger at apex	generally larger at apex	larger at apex
Paraphyses shape 2	undulated	undulated	undulated	undulated	straight	undulated
Hymenium thickness (μm)	(50–)**85**-<u>**120**</u>-**150**(–210)	70–84	(45–)**75**-<u>**95**</u>-**115**(–160)	(69.0–)<u>**80.5**</u>(–92.0)	(39–)**72**-<u>**76**</u>-**79**(–119)	(60–)**100-115**(–200)
Subhymenium thickness (μm)	(12–)**25**-<u>**38**</u>-**51**(–75)	40.8	(10–)**15**-<u>**27**</u>-**39**(–82)	(27.6–)<u>**31.0**</u>(–34.5)	(7–)**23**-<u>**27**</u>-**31**(–48)	—
Ascospore halo	rarely present	absent	present or absent	absent	absent	absent
Ascospore length (μm)	(7.5–)**10.0**-<u>**14.0**</u>-**18.5**(–25.0)	(7.2–)<u>**8.5**</u>(–9.6)	(8.0–)**11.5**-<u>**13.5**</u>-**15.5**(–21.5)	(9.7–)<u>**11.5**</u>(–13.8)	(10.5–)**14.0**-<u>**16.5**</u>-**17.0**(–20.5)	(15–)**17-20**(–22)
Ascospore width (μm)	(5.0–)**7.0**-<u>**9.0**</u>-**12.0**(–14.0)	(4.8–)<u>**5.7**</u>(–7.2)	(3.0–)**5.5**-<u>**7.0**</u>-**8.5**(–11.0)	(6.9–)<u>**7.2**</u>(–9.2)	(7.0–)**7.5**-<u>**9.0**</u>-**10.0**(–12.5)	(5–)**10-12**(–18)
Pycnidium diameter (μm)	(25–)**45**-<u>**90**</u>-**140**(–220)	—	(25–)**45**-<u>**55**</u>-**65**(–100)	—	(50–)<u>**72**</u>(–96)	—
Conidium length (μm)	(3.4–)<u>**5.6**</u>-**7.7**(–9.8)	—	(2.9–)**3.5**-<u>**5.1**</u>-**6.7**(–9.3)	—	<u>**4.8**</u>	(4–)**15-17**(–42)
Photobiont	*Trentepohlia* or trebouxioid	*Trentepohlia*	trebouxioid or *Trentepohlia*	*Trentepohlia*	trebouxioid	trebouxioid
Substrate reaction to HCl	negative or positive	negative	negative	negative	positive or negative	rarely positive (saxicolous species)
Distribution	arctic-alpine, rare in boreal zone and in Great Lakes region	Switzerland	boreal-hemiboreal subzone, temperate, and Appalachian Mountains; or arctic-alpine	boreal alpine (sensu Magnusson 1933)	arctic-alpine	widespread

all species which do not have the same type of asci as the type species of a genus 'A' should be transferred to another genus 'B' with the same type of ascus." If one accepts this principle and the resulting segregation of *Eiglera* from *Hymenelia* by Hafellner and uses monophyly as the grouping criterion when considering other species groups within *Hymenelia*, at least one of the three homogeneous groups within *Hymenelia* would have to be recognized as a separate genus as well (Figs. 5, 7A and 9A).

The problem of using ascal structure as revealed by Lugol's solution for classifying ascomycetes lies in our understanding of its evolution. The current usage of ascus apical structure for the classification of lichenized ascomycetes assumes, for example, that an ascus type is never lost or modified in one of the lineages within a monophyletic group. This premise needs to be investigated through phylogenetic studies before it is used to modify the classification of lichenized ascomycetes.

Photobiont Differences, Intergeneric Similarities, and Intrageneric Heterogeneity: The* Ionaspis epulotica-Hymenelia prevostii *Problem. Even if *Ionaspis epulotica* (Ach.) Blomb. & Forssell var. *epulotica* and *Hymenelia prevostii* (Duby) Kremp. (the type species of *Hymenelia*) are classified in separate genera based on photobiont differences, they are still morphologically very similar species within the *Ionaspis-Hymenelia* complex (Jørgensen 1989). In the present study, both species were found to be part of the homogeneous group *epulotica*. Magnusson's (1933) reasoning regarding the status of *Hymenelia prevostii* and *Ionaspis epulotica* s. str. was inconsistent. He compared *I. epulotica* var. *patellula* (Arnold) H. Magn. (= *I. epulotica* var. *epulotica*) with *H. prevostii*, and concluded that these taxa should be considered different species in different genera on the basis of the hyphal cell shape and stratification, and aggregation pattern and size of the algae. Nevertheless, Magnusson (1933) implied that *I. epulotica* and *H. prevostii* were easily confused, if not conspecific, when, in his discussion of the thallus of *Ionaspis*, he considered *I. prevostii* sensu Bachmann (1892, 1919) to be a synonym of *I. epulotica* var. *patellula*.

Six critical specimens were studied to verify the photobiont composition of both species and to determine whether any mycological differences could justify the genus and species rank given to each taxon. These specimens are the lectotype of *Gyalecta epulotica* Ach., which is the basionym of *Ionaspis epulotica*, and five specimens mentioned in the protologue for *Urceolaria prevostii* Duby, the basionym of *Hymenelia prevostii* (Table 7). The photobiont cell walls of the lectotype of *I. epulotica*, two specimens of *H. prevostii* distributed as Mougeot and Nestler exsiccatae, and a specimen of *H. prevostii* collected by Prost were all found to be refringent in polarized light (i.e., the photobiont was *Trentepohlia*). The two other syntypes of *H. prevostii* were found to be associated with *Trebouxia*, as was the material seen by Krempelhuber [not included in Table 7: (Germany), Bayern, Berchtesgadener Alpen, Watzmann, 5500-8000', 1855, *Krempelhuber 3468;* M (Herb. Krempelhuberi, 5)] and identified as *H. prevostii* f. *rosea* Kremp. Since specimens cited in the protologue of *Urceolaria prevostii* by Duby (1830) were found with one or the other photobiont, this implies that the generic distinction between *Ionaspis epulotica* and *Hymenelia prevostii* based solely on a difference in photobionts no longer holds.

Duby did not specify a holotype. A lectotypification is therefore necessary and crucial, since the selection of a specimen associated with *Trentepohlia* would eliminate the only known character used to separate the two genera as they were circumscribed prior to this study. The specimen selected here as the lectotype is associated with *Trentepohlia* (see the description of *Hymenelia* for the lectotypification). Since no significant differences in mycological characters were found (Table 7), we believe *Ionaspis epulotica* var. *epulotica* and *Hymenelia prevostii* are congeneric.

The question remains whether *Hymenelia epulotica* and *Hymenelia prevostii* should be maintained as distinct species. Jørgensen (1989) noted that *I. epulotica* s. str. may be confused with *H. prevostii* and that the only character distinguishing them is the same one used at the generic level, i.e., the photobiont. Using only one specimen from each species, Fröberg (1989) detected a different hymenial iodine reaction between *H. prevostii* (hymenium 0.3% I+ blue) and *I. epulotica* (hymenium 0.3% I+ red-brown). In the present study, the Lugol's reaction of the hymenium was not retained as a reliable character. Although we used 1.5% IKI (Lugol's) solution throughout this study (see Baral 1987; Common 1991), this character was found to be

TABLE 7. Comparison of critical material of *Ionaspis epulotica* var. *epulotica* (= *Hymenelia epulotica*) and *Hymenelia prevostii*.

Characters	*Hymenelia* (*Gyalecta*) *epulotica* (H-ACH) Angliae Lectotype	*Hymenelia* (*Biatora*) *prevostii* (M) Mougeot and Nestler exs. 848 Lectotype	*Hymenelia* (*Gyalecta*) *prevostii* (UPS) Galliae Le Prévost	*Hymenelia* (*Gyalecta*) *prevostii* (UPS) Galliae Mougeot	*Hymenelia* (*Biatora*) *prevostii* (CANL) Mougeot and Nestler exs. 848 Isolectotype	*Hymenelia* (*Urceolaria*) *prevostii* (M) Lozère Prost
Photobiont	*Trentepohlia*	*Trentepohlia*	*Trebouxia*	*Trebouxia*	*Trentepohlia*	*Trentepohlia*
Apothecia disk color (Kelly 1965)	(31) pale y. pink	(52) light orange	(52) light orange	(52) light orange	(73) pale orange yellow	(54) brownish orange
Average apothecia density (n/6.25 mm^2)	26.3	11.3	14.0	10.7	7.0	15.7
Apothecia disposition	agglomerate	agglomerate or not	agglomerate or not	agglomerate or not	agglomerate or not	agglomerate or not
Apothecia shape	round	subangular to irregular	irregular to elongate	irregular to elongate	round to subangular	irregular to elongate
Apothecia disk diameter (μm)	85.8–147.0	168.7–265.1	120.5–241.0	120.5–241.0	241.0–361.5	120.5–192.8
Apothecia margin thickness (μm)	24.5–49.0	36.2–48.2	24.1–48.5	24.1–36.2	48.2–72.3	36.1–48.2
Ascospore length (μm)	(12.8–)14.8(–16.0)	15.2	(12.5–)14.9(–18.8)	(15.0–)16.0(–17.5)	(12.5–)15.0	(13.8–)14.4
Ascospore width (μm)	(6.5–)8.7–11.2(–13.4)	(10.0–)11.4(–12.5)	10.2	10.6(–12.5)	10.4	12.0
Ascospore shape	subspherical to ellipsoid	subspherical to ellipsoid	subspherical to ellipsoid	subspherical to ellipsoid	subspherical to ellipsoid	subspherical to ellipsoid
Ascospore epispore	not halonate	not halonate	not halonate	not halonate	not halonate	not halonate
Ascospore disposition in ascus	uniseriate	uniseriate	uniseriate	uniseriate	—	uniseriate
Hymenium thickness (μm)	108	95	175	250	—	175
Subhymenium thickness (μm)	48	—	75	50	—	88
Excipulum proprium thickness (μm)	36	38	30	112	—	—
Paraphyses ramification	from the base to the apex	from the base to the apex	from the base to the apex	from the base to the apex	—	from the base to the apex
Paraphyses constriction	slightly constricted	slightly constricted	slightly constricted	not constricted	—	slightly constricted
Paraphyses shape	about the same width from top to bottom	about the same width from top to bottom	about the same width from top to bottom	about the same width from top to bottom	—	about the same width from top to bottom
Paraphyses degree of fusion	highly anastomosed	highly anastomosed	highly anastomosed	highly anastomosed	highly anastomosed	highly anastomosed

difficult to interpret because intermediate reactions were seen, where only some part(s) of the hymenium reacted. Also, most species had both positive and negative reactions. Finally, this character was never shown to be important in the explanation of the clusters by the PCA and CVA. A more extensive study using different iodine solutions (Common 1991) and also including *I. similis* (A. Massal.) Poelt & Vězda is still needed. In comparing type material of *Hymenelia prevostii* associated with trebouxioid algae or *Trentepohlia* with the lectotype of *Hymenelia epulotica*, no characters were found to suggest any distinction between these taxa at the species level (see Table 7). We therefore regarded the names as synonymous, with *H. epulotica* having priority (see description of *Hymenelia* below).

Classification of the Hymenelioid Lichens. Körber (1855) originally circumscribed the Hymeneliaceae on the basis of a "pseudogymnocarpic" apothecial development and a double excipulum, forming a link between *Lecanora* and *Lecidea* Ach., and including *Hymenelia*, *Petractis* Fr. and *Thelotrema* Ach. In a recent listing of the families and genera of ascomycetes, Eriksson and Hawksworth (1993) included nine genera in the Hymeneliaceae, including *Aspicilia*. Hafellner (1984) regarded the Hymeneliaceae as a "still poorly understood family," and included the Aspiciliaceae only doubtfully. Later, Hafellner (1989) excluded *Aspicilia* and closely related genera from the Hymeneliaceae. Our study did not include all critical taxa that need to be considered to determine the phylogenetic relationships and classification of *Aspicilia*. Therefore, we prefer to keep the genus *Aspicilia* within the Hymeneliaceae.

The grouping of the *epulotica, haematina,* and *melanocarpa* homogeneous groups and of the *alba, lacustris,* and *odora* homogeneous groups into two distinct monophyletic groups was supported by both allozyme and the combined data sets (Figs. 7A and 9A). These results contradict previous classifications of the hymenelioid lichens with respect to the *epulotica* and *odora* groups. Magnusson (1933) included both the *odora* and *epulotica* groups under the sect. *Pallescentes* H. Magn. of *Ionaspis*. Our results suggested that the *odora* group is more closely related to both the *lacustris* and *alba* groups than to the *epulotica* group and that all pale apothecial disks are not homologous. There is no solid basis for recognizing two subgeneric entities within *Hymenelia* based on disk color.

The reclassification suggested by the present study of species groups classified under *Hymenelia* and *Ionaspis* in relation to *Eiglera* and *Aspicilia*, is summarized in Fig. 10. All species groups previously classified under *Ionaspis*, except for the *suaveolens* group (which includes the type of *Ionaspis*), and the *odora* group (see below), were reclassified under *Hymenelia*. *Ionaspis* would then include the *suaveolens* group, including *I. suaveolens, I. fuscoclavata, I. granvina,* and *I. handelii* (all species with an HNO_3 negative and KOH negative epihymenium); the *odora* group, including *I. alpina, I. lavata, I.* sp.# 1 (Lutzoni 1990), *I. odora,* and *I. ventosa* (all species with an HNO_3+ orange and KOH+ violet epihymenium); the *lacustris* group (*Hymenelia lacustris*); and the *alba* group, a new species of *Ionaspis* recently described (Lutzoni 1994). This circumscription of *Ionaspis* will be valid as long as *I. suaveolens* is considered to be sufficiently related to the *odora* group to be included in the same genus. Finally, the generic circumscriptions of *Aspicilia* and *Eiglera* in the modern sense (Eriksson and Hawksworth 1993) were not changed in this study, although the generic status of *Eiglera* is put into question.

Taxonomic Treatment

1. Ascus apex IKI+. ..... 3. *Eiglera*
1. Ascus apex IKI-.
   2. Epihymenium olive green to olive brown, remaining this color in HNO_3; conidia mostly more than 15 μm long; thallus usually thick, epilithic, verrucose to areolate; typical lichen secondary metabolites including β-orcinol depsidones can be present ..... *Aspicilia*
   2. Epihymenium hyaline or with pigments different from above; conidia under 10 μm long; thallus mostly thin, epi- or endolithic; continuous to rimose-areolate; typical lichen secondary metabolites including β-orcinol depsidones absent except in *Porpidia pseudomelinodes* Schwab that has been recently transferred to *Hymenelia* by Gowan and Ahti (1993) as *H. ochrolemma* (Vain.) Gowan & Ahti.
      3. Apothecial disk yellowish brown or grayish to almost black or whitish or rusty brown, the rusty brown color due to the presence of an epipsamma; when apothecial pigment present (not epipsam-

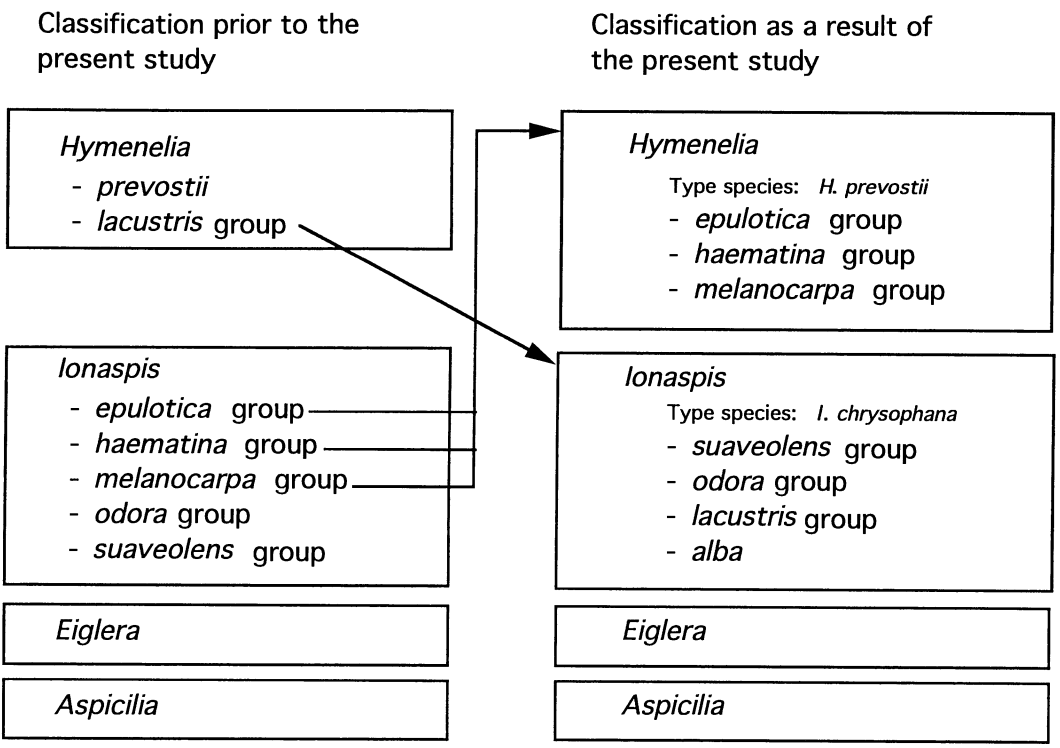


FIG. 10. Schematic summary of the rearrangements in the classification of the hymenelioid fungi as suggested by anatomical-morphological and allozyme data in this study.

ma), HNO_3+ orange yellow, KOH+ dark violet; thallus epilithic only .... 2. *Ionaspis* p.p. (*alba, lacustris,* and *odora* groups)

3. Apothecial disk black or pinkish, no epipsamma present; when black, apothecial pigment HNO_3+ violaceous pink, or + intensified green, KOH-; thallus epi- or endolithic.
   4. Apothecial disk black or pinkish; if black, epihymenium HNO_3+ violaceous pink; hymenium (50–)**85**–**120**–**150**(–210) μm thick ..... 1. *Hymenelia*
   4. Apothecial disk black; epihymenium HNO_3 negative or intensified green; hymenium (50–)**70**(–92) μm thick .. .... 2. *Ionaspis* p.p. (*suaveolens* group)

HYMENELIACEAE Körb. em. Hafellner, Beih. Nova Hedwigia 79: 348. 1984. "Hymenelieae" Körb., Syst. lich. Germ. 327. 1855.—TYPE GENUS: *Hymenelia* Kremp.

1. HYMENELIA Kremp., Flora 35: 24. 1852.—TYPE SPECIES: *Hymenelia prevostii* (Duby) Kremp.—*Biatora prevostii* Fr. in Moug.& Nestl., Stirpes Crypt. exs. 848. 1826. (nom. nud.).—*Urceolaria prevostii* Duby, Bot. gall. 671. 1830. (nom. illegit. superfl.).—*Gyalecta prevostii* Fr., Lichenogr. eur. reform. 197. 1831.—*Lecidea prevostii* (Fr.) Schaer., Lich. Helv. Spic. 179. 1833.—*Hymenelia prevostii* (Fr.) Kremp. α *rosea* Kremp., Flora 35: 25. 1852.—TYPE: (France), "circa Rothomagum," *Mougeot & Nestler*: Stirpes Crypt... no. 848 sub "*Biatora Prevostii* Fries in Litt." (Lectotype here designated: M!; isolectotype: CANL!). [=*Hymenelia epulotica* (Ach.) Lutzoni.]

Pinacisca A. Massal., Neagen. lich. 5. 1854 (fide Poelt and Vězda 1981).—TYPE SPECIES: *P. similis* A. Massal.

Manzonia Garov., Manzonia 97. 1866. (Mem. Soc. Ital. Sci. nat 2[8]) (fide Poelt and Vězda 1981).—TYPE SPECIES: *M. cantiana* Garov.—*Hymenelia prevostii* γ *caerulescens* Kremp., Flora 35: 25. 1852.

Thallus very variable in color, from light gray to pale yellow, or grayish yellowish brown to orange yellow (31, 32, 69, 70, 73, 76, 79, 80, 89, 92, 93, 265), epi- or endolithic when on calcareous rocks, rimose and/or rimose-areolate when

epilithic. Photobiont *Trentepohlia* or trebouxioid algae. Apothecia circular to irregular, density (1.7–)**4.1-8.7-13.3**(–27.0) /6.25 mm²; disk (0.07–)**0.10-0.25-0.46**(–0.72) mm diameter, black or pale yellowish in old herbarium specimens, pinkish if recently collected; margins (0–)**30-80-150**(–290) μm thick, not prominent to slightly prominent when young, becoming slightly prominent to prominent, rarely becoming very prominent and constricted at the base (epilithic individuals only). Lateral excipulum proprium (0.0–)**32-65-97**(–225) μm thick, hyaline to reddish black (24), dark olive brown (96), dark grayish olive (111), blackish green (152), very dark bluish green (166), or bluish black (193), HNO_3+ violaceous pink or negative, KOH negative, textura extremely variable. Basal excipulum proprium (0.0–)**11-18-24**(–31) μm thick, hyaline to grayish yellowish brown (80), dark grayish olive (111), very dark green (147), or hyaline or olive black (114), or very dark bluish green (166), or dark grayish brown to brownish black (62, 65), HNO_3+ violaceous pink or negative, KOH negative, textura very variable. Hypothecium **0-28-59**(–225) μm thick, hyaline, HNO_3 and KOH negative, textura globulosa, or angularis, or epidermoidea. Subhymenium (12–)**25-38-51**(–75) μm thick, hyaline, IKI+ blue or negative, HNO_3 and KOH negative. Hymenium (50–)**85-120-150**(–210) μm thick, hyaline or concolorous with epihymenium, IKI+ blue or negative; pigmented part (30)-**46-60**(–77) μm thick, HNO_3+ violaceous pink or negative, KOH negative. Epihymenium bluish green (161, 165, 166), or olive green to brownish black, olive black, or blackish green (65, 96, 114, 128, 146, 147, 151, 152), or hyaline, HNO_3+ violaceous pink or negative, KOH negative. Epipsamma absent, color of the disk given by pigments in the ascoma. Paraphyses undulated, dichotomously branched at the apex, usually with ramification below the apex, not constricted to moniliform (including submoniliform), anastomosed; cells the same thickness from top to bottom, or larger at the apex. Ascus tip IKI negative. Ascospores (7.5–)**10.0-14.0-18.5**(–25.0) × (5.0)**7.0-9.0-12.0**(–14.0) μm, hyaline, simple, not or rarely halonate, uniseriate or aseriate, 8 per ascus. Pycnidia (25–)**45-90-140**(–220) μm diameter, concolor with disks, buried in thallus. Pycnidiospores (3.4–)**5.6-7.7**(–9.8) × 1.0(–2.0) μm, bacilliform.

Habitat.—Mainly submerged in small creeks, rivers, or growing in the spray zone of falls. Also growing in intermittent creeks, on wet cliffs bordering rivers or on rocky shores of lakes. Some species, however, are found in extremely dry habitats such as the summit of small hills exposed to strong winds, on calcareous rock only. When found in wet habitats, they colonize siliceous or calcareous rocks.

Distribution.—Arctic-alpine, extending south to the southern Rocky Mountains, with an intrusion into the boreal zone. One taxon is found in the temperate zone of the Great Lakes region.

Species Included.—**Hymenelia arctica** (Lynge) Lutzoni, comb. nov. ≡ *Ionaspis arctica* Lynge, Lich. Nov. Zemlya 43. 1928.

H. carnulosa (Arnold) Lutzoni, comb. nov. ≡ *Aspicilia carnulosa* Arnold, Flora 1869: 267. 1869. = *Ionaspis carnosula* (Arnold) Arnold

H. coerulea (DC.) A. Massal.

H. cyanocarpa (Anzi) Lutzoni, comb. nov. ≡ *Aspicilia cyanocarpa* Anzi, Manipulus 145. 1862. = *Ionaspis cyanocarpa* (Anzi) Th. Fr. ex Jatta.

H. epulotica (Ach.) Lutzoni, comb. nov. ≡ *Gyalecta epulotica* Ach., Lich. Univ. 151, tab. 1, fig. 8. 1810. ≡ *Ionaspis epulotica* (Ach.) Blomberg & Forssell–TYPE: "Anglia, *Harriman 15*" (Lectotype here designated: H-Ach 57!; isolectotypes: BM, UPS!). = *H. prevostii* (Duby) Kremp. (See the section of the discussion entitled "Photobiont differences, intergeneric similarities, and intrageneric heterogeneity: The *Ionaspis epulotica-Hymenelia prevostii* problem.")

H. fuegensis (P. M. Jörg. & R. Sant.) Lutzoni, comb. nov. ≡ *Ionaspis fuegensis* P. M. Jörg. & R. Sant., Norw. J. Bot. 9: 431. 1989.

H. haematina (Körb.) Lutzoni, comb. nov. ≡ *Aspicilia haematina* Körb., Parerga lichenol. 100. 1860. = *Ionaspis haematina* (Körb.) Th. Fr., comb. inval.

H. heteromorpha (Kremp.) Lutzoni, comb. nov. ≡ *Aspicilia cinereorufescens* γ *heteromorpha* Kremp., Lich.-Fl. Bayerns 175. 1861. = *Ionaspis heteromorpha* (Kremp.) Th. Fr. ex Arnold = *I. annularis* H. Magn. (fide Santesson 1993). = *I. ochracella* (Nyl.) H. Magn. (fide Santesson 1993). = *I. reducta* H. Magn. (fide Santesson 1993). = *I. schismatopis* (Nyl.) Hue (fide Santesson 1993).

H. melanocarpa (Kremp.) Arnold

H. similis (A. Massal.) M. Choisy.

H. rhodopis (Sommerf.) Lutzoni, comb. nov. ≡ *Lecanora acharii* Sommerf. var. *rhodopis* Som-

merf., Suppl. Fl. lapp. 88. 1826. = *Ionaspis rhodopis* (Sommerf.) Blomberg & Forssell. = *I. ochromicra* (Nyl.) Hue (fide Santesson 1993).

Species with Uncertain Status.—Ionaspis aigneri Zahlbr.

Two questions must be answered here with respect to the type: 1) what is the type species of *Hymenelia*, and 2) what is the type specimen of that species? Eigler (1969: 155) cited "*Lecanora coerulea* (DC.) Nyl." as the type species of *Hymenelia*, but *Hymenelia* was established as a monotypic genus containing only *H. prevostii*. This species, in its strict sense, must, therefore, be considered the type species of the genus. *Lecanora coerulea* was only one of several varieties of *H. prevostii* mentioned by Krempelhuber (1852) in the protologue and, therefore, must be rejected as the type species. *Hymenelia prevostii* sensu Krempelhuber (1852) is mainly an endolithic lichen, with very polymorphic thalli and apothecia. These characters along with anatomical characteristics distinguished this taxon from *Lecidea* Ach., *Gyalecta* Ach., *Biatora* Fr., and *Thelotrema* Ach.

Krempelhuber's concept of *Hymenelia prevostii* was quite broad since he included four main infraspecific entities under that name, all of which are now treated at the species level or as stirps: 1) α *rosea*, which he chose as the typical form of the species, and which is very similar to *Hymenelia epulotica*; 2) β *melanocarpa* a. *punctata*, which correponds to Magnusson's (1933) epilithic *Ionaspis* sect. *Cærulescentes* H. Magn. and to the *haematina* group in this treatment; 3) β *melanocarpa* b. *lecanorina*, which corresponds to *H. melanocarpa*, and 4) γ *caerulescens*, which is now called *Hymenelia coerulea*. The protologue for the genus *Hymenelia*, therefore, included almost all the taxa that were, until now, included in the genus *Ionaspis* and contained taxa mostly associated with *Trentepohlia*, except for *H. coerulea* and a fraction of the individuals of *H. epulotica*. Krempelhuber (1852) did not consider the photobiont difference a diagnostic character for this genus. Since *Hymenelia prevostii* (Duby) Kremp. s. str. has to be the type species of the genus, and since, in the protologue, Krempelhuber (1852) clearly stated that α *rosea* is the "forma typica," we have rejected Eigler's typification with γ *caerulescens* and replaced it with α *rosea*.

In the same paper, Krempelhuber stated that it was Fries (1831: 197), using Le Prévost's specimens, who first described *H. prevostii*, but in the genus *Gyalecta*. The species, however, had already been validly published by Duby in 1830 under the name *Urceolaria prevostii* where Le Prévost's specimens (and others) were cited. The latter name is considered here to be the basionym of *H. prevostii* (Duby) Kremp., in agreement with Erikkson and Hawksworth (1993) and Santesson (1984, 1993). Farr et al. (1979) and Poelt and Vězda (1981) considered *Gyalecta prevostii* Fr. (1831) the basionym, whereas Hafellner (1984), gave "(Schaerer) Kremplh." as the authors of *Hymenelia prevostii*, although Schaerer (1833) himself cited *Gyalecta prevostii* Fr. as the basionym. There was even a previous name for *U. prevostii* Duby, that is, *Biatora prevostii* Fr., found on the label of Mougeot and Nestler exs. no. 848 and cited by Duby (1830) and Schaerer (1833). Yet it was never validly published, as noted by Duby himself as well as by Schaerer (1833). No diagnosis was found for *B. prevostii* Fr. either on the label of the exsiccata or in any accepted printed matter (ICBN, Art. 29). The latter name is a *nomen nudum* (ICBN Art. 32).

Because the epithet "*prevostii*" was used in three different genera within three years, the typification of *Urceolaria prevostii* Duby was somewhat complicated. In UPS, a specimen identified by Fries as *Gyalecta prevostii* and collected by Le Prévost in "Galliae" was assigned the status of holotype. It cannot be a type, however, because there is already a reference to Fries' publication on the label ("L.E. p. 197") undoubtedly referring to Lichenographia Europaea, page 197, where Fries' description of *Gyalecta prevostii* appears, and therefore probably collected after 1831. Duby (1830) notes three localities in the protologue of *Urceolaria prevostii*: "Ad rupes calcarias Jurassi (cl. Moug.et Nestl.), Rothomagi (cl. Le Prév.), Mimatis (cl. Prost). -Moug. et Nestl. vog. n. 848. *Biatora Prevostii* Fr. ined. ex cl. Le Prév. in litt. (v. s.)." All three localities are mentioned on the Mougeot and Nestler label of exsiccata 848. Since both Duby (1830) and Fries (1831) refer to this exsiccata, it seems clear that this material should serve as a type and a lectotypification is necessary.

Duby's lichen collection is mainly in STR, but some specimens can be found in UPS (Hawk-

sworth 1974). Ideally, a lectotype should be selected from Mougeot and Nestler material from one of these herbaria. Unfortunately, it was not possible to see any material from STR, and it was found that the Mougeot and Nestler exsiccata no. 848 in UPS was not *Hymenelia prevostii* but rather *Petractis clausa* (Hoffm.) Kremp. Two other packets of the exsiccata (from M and CANL) were examined by the first author, however, and both fit the description in the protologue. We have, therefore, selected the specimen in München (M) as the lectotype.

2. IONASPIS Th. Fr., Lichenogr. Scand. 273. 1871. Emend. Lutzoni & Brodo.—TYPE SPECIES: *Ionaspis chrysophana* (Körb.) Th. Fr. ex Stein, Flecht. Cohn's Krypt.-Fl. Schl. Vol. 2, 2. 151: 1879, designated by Clements and Shear (321: 1931).—*Aspicilia chrysophana* Körb., Syst. lich. Germ. 159: 1855. ≡ (by proposed conservation of type, Lutzoni & Brodo, Taxon 43: 657. 1994) *I. suaveolens* (Fr.) Th. Fr. ex Stein.—TYPE: Körber Typenherbar, Sudeten, *Körber 12* (lectotype in Lutzoni & Brodo, Taxon 43: 657. 1994: L!).

Thallus pinkish white (9), pale yellowish pink (31), grayish brown (62, 64), or yellowish white to dark grayish yellow (90-93), or light orange to strong brown (52, 55, 57), or pale to moderate orange yellow (70, 71, 73), or light to deep yellowish brown (74-77, 79, 80), or light olive gray (112), epilithic, rimose and/or rimose-areolate. Photobiont trebouxioid or *Trentepohlia*. Apothecia circular, subangular or irregular, (2.8-)**7.8-16.5-25.2**(-42.2) /6.25 mm^2; disk (0.03-)**0.2-0.4**(-0.7) mm diameter, grayish reddish orange to reddish brown (39-41, 43, 45-47), or light to deep orange (50-54), or light grayish brown to deep brown (55-64), or pale to dark orange yellow (69, 72, 73), or light grayish to deep yellowish brown (75, 76, 79, 80, 81), or white to yellowish to dark gray (92, 93, 263, 266); or (in *Ionaspis suaveolens*) black (267); margins (0-)**30-60-100**(-270) μm thick, not prominent to slightly prominent when young, remaining this way or becoming slightly prominent to prominent. Lateral excipulum proprium (0.0-)**25-48-75**(-150) μm thick, hyaline or vivid to deep orange or orange yellow (48, 51, 66, 68, 69), or strong brown to brownish black (55, 59, 65), or yellowish brown (74-76), or vivid yellow (82), or light olive brown (94), or very dark green (147), and HNO_3+ orange yellow (*odora* group), or intensifying the dark green color to dark blue (*suaveolens* group), or negative (*alba* and *lacustris* groups), and KOH+ dark violet (*odora* group), or negative (*suaveolens, alba,* and *lacustris* groups); textura prismatica or epidermoidea. Basal excipulum proprium (0-) **12-21**(-37.5) μm thick, mostly hyaline or light to grayish yellowish brown (76, 80), HNO_3 and KOH reactions same as lateral excipulum proprium, textura mostly prismatica or oblita. Hypothecium (0-)**11-38-64**(-123) μm thick, hyaline or pale yellowish brown, HNO_3 and KOH negative, textura mostly globulosa, angularis or prismatica. Subhymenium (10-)**15-27 39**(-82) μm thick, hyaline, IKI+ blue or negative, HNO_3 and KOH negative. Hymenium (45-)**75-95-115**(-160) μm thick, (*odora* group) light to orange yellow (70, 72), or light to strong yellowish brown (74, 76), light grayish yellowish brown (79), grayish to dark yellow (88, 90), dark grayish olive (111) or hyaline, (*suaveolens* group) very dark green (147), (*alba* and *lacustris* groups) hyaline, rarely IKI+ blue, HNO_3 and KOH reactions same as lateral excipulum proprium. Epihymenium concolor with hymenium, HNO_3 and KOH reactions same as lateral excipulum proprium. Epipsamma present or not, responsible for the color of the apothecial disk of *lacustris* group and sometimes at the margin of the apothecia of the *alba* group. Paraphyses simple, or dichotomously branched at the apex, sometimes with ramification below the apex, slightly constricted, submoniliform or moniliform, anastomosed; cells generally larger at the apex. Ascus tip IKI negative. Ascospores (8.0-)**11.5-13.5-15.5**(-21.5) × (3.0-)**5.5-7.0-8.5**(-11.0) μm, hyaline, simple, densely halonate (character sometimes difficult to see) or not halonate, uniseriate or aseriate, 8 per ascus. Pycnidia (25-)**45-55-65**(-100) μm diameter, concolor with disk, buried in thallus (pycnidia not seen on lectotype). Conidia (2.9-)**3.5-5.1-6.7**(-9.3) × 1.0 μm, bacilliform or filiform.

Habitat.—Submerged in small creeks, rivers, or growing in the spray zone of falls. Found also on rocky shores of lakes, on boulders in forest openings, or on small boulders in deciduous forests. Colonized rocks are siliceous.

Distribution.—Boreal-hemiboreal subzone, temperate, and Appalachian Mountains; or Arctic-alpine.

Species included.—*I. alba* Lutzoni, The Bryologist 97: 393-395, 1994.

Ionaspis alpina Zahlbr.

I. fuscoclavata Eitner

I. granvina P. M. Jörg. & R. Sant.

I. handelii Zahlbr.

I. lacustris (With.) Lutzoni, comb. nov. ≡ *Lichen lacustris* With., Arr. Brit. pl. ed. 3, 4:21, tab. XXXI., fig. 5. 1796. = *Hymenelia lacustris* (With.) M. Choisy.—TYPE: *"Griffith"* (Holotype: Herbarium Withering no. 66, BM!).

I. lavata H. Magn.

I. odora (Ach.) Th. Fr. ex Stein

I. ventosa P. M. Jörg. & R. Sant.

I. sp. # 1 (sensu Lutzoni 1990).

I. suaveolens (Fr.) Th. Fr. ex Stein ≡ *Aspicilia chrysophana* Körb., Syst. lich. Germ. 159: 1855. (by proposed conservation of type, Lutzoni & Brodo, Taxon 43: 657. 1994).

Species with uncertain status.—*Hymenelia ceracea* (Arnold) Poelt & Vězda

The genus *Ionaspis* was established by Th. Fries in 1871. His description of the genus is very short and is found under *Aspicilia* as follows: "-E speciebus antea Aspiciliis adscriptis permultæ (v. c. *chrysophana* Körb., *rhodopis* Sommerf., *odora* Ach., *suaveolens* Ach., *hæmatina* Körb., *cyanocarpa* Anzi, *epulotica* Arnold exs. 41 et 164, *cinereorufescens β heteromorpha* Krmplh. cet.) ob gonidia concatenata sunt excludendæ; ex his novum genus, *Ionaspis* Th. Fr., est condendum." The chainlike alga cited by Th. Fries is *Trentepohlia*, and it is the only diagnostic character. Fröberg (1989) and Santesson (1984) concluded that this description did not fulfill the requirements of the International Code of Botanical Nomenclature based on the ICBN Article 13.1(d), stipulating that "for nomenclatural purposes names given to lichens shall be considered as applying to their fungal component." However, since at the time the genus *Ionaspis* was described, characters of the photobiont were considered as good as any other characters for lichen classification, Th. Fries' description of *Ionaspis* should be regarded as validly published, in agreement with Cannon et al. (1985), Jørgensen (1989), and Santesson (pers. comm. 1989), unless it is decided that Article 13.1(d) is retroactive to that time. Th. Fries is regarded by Cannon et al. (1985) as the combining author of *chrysophana* and *suaveolens* within *Ionaspis* at the species level. Since Th. Fries never actually used these combinations in that publication (see Art. 33.1, ICBN), we are inclined to agree with Jørgensen (in litt.) and Santesson (1984) in regarding these combinations not to have been made until they were listed by Stein (1879).

No type species was designated by Th. Fries (1871) for the genus *Ionaspis*. Because of the heterogeneity of the taxa originally placed in the genus, the typification of the genus is especially critical for its circumscription. Eigler (1969) erroneously proposed *I. ceracea* (Arnold in Kremp.) Jatta as the type for *Ionaspis*. This species is not part of the original description. Recently, Hafellner (1984) chose *I. epulotica* (Ach.) Th. Fr. as the lectotype, overlooking the fact that *I. chrysophana* had already been chosen as the lectotype of *Ionaspis* by Clements and Shear (1931, sub *"Jonaspis chrysophana* (Kbr.) Stein" p. 321).

The next problem, however, was finding exactly how to apply the name *chrysophana*. *Ionaspis chrysophana* was long considered to be a synonym of *I. suaveolens*, but a careful typification has shown the situation to be very complex (Lutzoni and Brodo 1994). The basionym of *suaveolens* is *Gyalecta suaveolens* Fr., and a strict application of the type principle would require that the name *suaveolens* be taken up for the well-understood taxon *I. odora* in a way that would completely reverse the traditional use of both names. For this reason, we have proposed to conserve the name *Gyalecta suaveolens* Fr. with a conserved type which would maintain the current usage of *I. odora* and *I. suaveolens* (Lutzoni and Brodo 1994).

3. EIGLERA Hafellner, Beih. Nova Hedw. 79: 276. 1984.—TYPE SPECIES: *Eiglera flavida* (Hepp) Hafellner.—*Lecanora flavida* Hepp, Abbild. Beschr. Spor. no. 630. 1860.—TYPE: "An erratischen Verrucano = Blöcken, auf dem Albis K.Z. Dr. Hegetchweiler No. 1158, Lich. helvet. exs. Schaer. et Hepp."

Thallus mainly light grayish yellowish brown to grayish yellowish brown (79–80), dark grayish yellow to yellowish gray (91–93), light bluish gray (190), or light gray (264), epi- or endolithic, continuous or rimose to rimose-areolate when epilithic. Apothecia circular to subangular or irregular, density (5.3–)**20**(–66) /6.25 mm^2; disk (0.14–)**0.21–0.34–0.48**(–0.89) mm diameter, black; margins (19–)**45–80–100**(–250) μm

thick, mostly slightly prominent, rarely not prominent, or prominent when young, sometimes becoming very prominent and constricted at the base when mature (excluding endolithic individuals). Lateral excipulum proprium (8–)**34-39-43**(–102) μm thick, greenish black, very dark bluish green, blackish purple to bluish black (157, 165, 166, 188, 193, 230), HNO_3+ violaceous pink, KOH negative, textura prismatica or porecta. Basal excipulum proprium (0–)**6-11-13**(–24) μm thick, hyaline or very dark red (17) or dark grayish red to brownish black (20, 65), HNO_3 and KOH negative, textura very variable. Hypothecium (0–)**26-28-30**(–63) μm thick, hyaline, rarely dark grayish red (20), HNO_3 and KOH negative, textura globosa, angularis, or epidermoidea. Subhymenium (7–)**23-27-31**(–48) μm thick, hyaline, very rarely dark red (17), mostly IKI+ blue, HNO_3 and KOH negative. Hymenium (39–)**72-76-79**(–120) μm thick, hyaline or concolorous with epihymenium, mostly IKI+ blue; pigmented part (0–)**25-27-29**(–51) μm thick, HNO_3+ violaceous pink, KOH negative. Epihymenium dark reddish to dark olive brown (44, 78, 96), blackish green to greenish black (152, 157), or moderate bluish green to bluish black (160, 161, 164, 193), HNO_3+ violaceous pink, KOH negative. Epipsamma absent; color of the disk given by pigments in the ascoma. Paraphyses straight, simple or branched only at the tip, rarely branching lower, moniliform or with or without constrictions, with few anastomoses; cells usually wider at the apex. Ascus tip IKI+ blue, and apical cap IKI+ bluish brown. Ascospores (10.5–)**14.0-16.5-17.0**(–20.5) × (7.0–)**7.5-9.0-10.0**(–12.5) μm, hyaline, simple, not halonate, aseriate, 8 per ascus. Pycnidia (50–)**72**(–96) μm diameter, buried in thallus. Pycnidiospores **4.8** × 0.8–0.9 μm, bacilliform.

Habitat.—In brooks or rivers, or on cliff faces and boulders. On calcareous or siliceous rocks.

Distribution.—Arctic-alpine.

Species included.—*Eiglera flavida* (Hepp) Hafellner.

E. homalomorpha (Nyl.) Clauzade & Cl. Roux.

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APPENDIX 1. Annotated list of 61 characters used in the anatomical-morphological study. The 35 characters used in the statistical procedure to delimit homogeneous groups within the *Ionaspis–Hymenelia* complex are preceded by a variable descriptor. The variable descriptors are as follows: (1) dichotomy, (2) alternative, (3) multistate unordered, and (4) multistate ordered of quantitative (Lefkovitch 1981).

Variable descriptors	Characters	States
	Thallus	
	—color	chart of centroid colors (Kelly 1965)
2	—type	1 = endolithic; 2 = epilithic
2	—photobiont	1 = trebouxioid; 2 = *Trentepohlia*
	Disk (mature apothecia)	
3	—color	chart of centroid colors (Kelly 1965)
4	—minimum diameter	μm
4	—maximum diameter	μm
	Apothecia	
	—shape	1 = circular; 2 = subangular; 3 = irregular; 4 = elongate
	—margin shape (immature apothecia, dry and epilithic thallus)	1 = not prominent; 2 = slightly prominent; 3 = prominent; 4 = very prominent and constricted at the base
3	—margin shape (mature apothecia, dry and epilithic thallus)	1 = not prominent; 2 = slightly prominent; 3 = prominent; 4 = very prominent and constricted at the base
4	—minimum margin thickness	μm
4	—maximum margin thickness	μm
4	—density	number of apothecia in 2.5 × 2.5 mm
3	—epihymenial color	chart of centroid colors (Kelly 1965)
2	—dark excipulum proprium	1 = continuous below the subhymenium; 2 = present in the apothecial margin only
1	—epipsamma	1 = present; 2 = absent
	—hymenial color	chart of centroid colors (Kelly 1965)
	—lateral excipulum proprium color	chart of centroid colors (Kelly 1965)
	—basal excipulum proprium color	chart of centroid colors (Kelly 1965)
	—minimum hymenial color thickness	μm
	—maximum hymenial color thickness	μm
4	—hymenial thickness	μm
4	—subhymenial thickness	μm
4	—hypothecial thickness	μm
4	—minimum lateral excipulum proprium thickness	μm
4	—maximum lateral excipulum proprium thickness	μm
	—minimum basal excipulum proprium thickness	μm
	—maximum basal excipulum proprium thickness	μm
	—hypothecial textura (Korf 1958)	1 = globulosa; 2 = angularis; 3 = prismatica; 4 = intricata; 5 = epidermoidea; 6 = oblita; 7 = porrecta
	—lateral excipulum proprium textura (Korf 1958)	1 = globulosa; 2 = angularis; 3 = prismatica; 4 = intricata; 5 = epidermoidea; 6 = oblita; 7 = porrecta
	—basal excipulum proprium textura (Korf 1958)	1 = globulosa; 2 = angularis; 3 = prismatica; 4 = intricata; 5 = epidermoidea; 6 = oblita; 7 = porrecta

APPENDIX 1. Continued

Variable descriptors	Characters	States
3	—paraphysal ramification	1 = simple; 2 = ramified below the apex; 3 = dichotomously ramified at the apex
3	—paraphysal constrictions	1 = none; 2 = slightly constricted; 3 = submoniliform; 4 = moniliform
2	—paraphyses shape 1	1 = larger at the apex; 2 = uniform in width
	—paraphyses shape 2	1 = straight; 2 = undulate
	—paraphysal anastomosis	1 = present; 2 = absent
1	—hymenial reaction to HNO_3	1 = positive; 2 = negative
1	—hymenial reaction to KOH	1 = positive; 2 = negative
1	—hymenial reaction to 1.5% IKI (Lugol's) solution	1 = positive; 2 = negative
	—subhymenial reaction to 1.5% IKI (Lugol's) solution	1 = positive; 2 = negative
1	—tholus reaction to 1.5% IKI (Lugol's) solution	1 = positive; 2 = negative
	Ascospores	
4	—minimum length	μm
4	—maximum length	μm
4	—average length	μm
4	—minimum width	μm
4	—maximum width	μm
4	—average width	μm
1	—halo	1 = present; 2 = absent
3	—organization in ascus	1 = uniseriate; 2 = biseriate; 3 = aseriate
	Pycnidia (part visible at the surface of the thallus)	
	—minimum diameter	μm
	—maximum diameter	μm
	—average diameter	μm
	Conidia	
	—minimum length	μm
	—maximum length	μm
	—average length	μm
	—minimum width	μm
	—maximum width	μm
	—average width	μm
	—shape	1 = bacilliform; 2 = rubanate; 3 = elliptic; 4 = filiform
	Ecological and phytogeographical data	
2	—substrate reaction to HCl	1 = negative; 2 = positive
3	—habitats	1 = wet cliffs; 2 = submerged or just above water in flowing water; 3 = intermittent creek (dry at the moment of collection); 4 = scree talus; 5 = dry fellfield; 6 = boulders in deciduous forests; 7 = falls sprayed zone; 8 = rocky lake shore
3	—bioclimatic zones	1 = arctic; 2 = boreal; 3 = hemiboreal subzone; 4 = temperate; 5 = subtropical to tropical; 6 = Appalachian mountains; 7 = northwestern Cordillera; 8 = southern Rocky Mountains; 9 = Sierra Nevada and American coastal mountains