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The genus *Anthracoidea* (*Basidiomycota*, *Ustilaginales*): a molecular phylogenetic approach using LSU rDNA sequences*

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The phylogenetic relationship of 52 specimens representing 30 species of *Anthracoidea* (*Ustilaginales*) was investigated by molecular analyses using sequence data from the large subunit (LSU) of nuclear rDNA. Phylogenetic trees were inferred with neighbour-joining (NJ), maximum parsimony (MP), and Bayesian Markov chain Monte Carlo (MCMC) methods. The results are discussed with respect to the species concept and the subdivision of the genus into subgenera and sections. Collections from different hosts and localities were compared. Our analyses can neither support nor significantly reject the hypothesis of the bipartition of the genus *Anthracoidea*. Thus, the representatives of the subgenus *Proceres* appeared in the NJ analysis as a moderately supported monophylum, whereas MCMC analysis revealed a polyphyletic topology for this group. Paraphyly of the subgenus *Anthracoidea* was supported by all methods used. Sections *Echinosporae* and *Leiosporae* were each represented by two species in our analyses which grouped together with high support. Section *Anthracoidea* should be restricted to a highly supported group with extremely irregular to angular teliospore shape. However, these three sections do not cover the whole diversity of the subgenus *Anthracoidea*. Molecular data largely supported the traditional circumscription of species, and species delimitations are discussed.

INTRODUCTION

All species of *Anthracoidea* can easily be recognized by the production of single teliospores in conspicuous black sori in the ovaries of plant hosts belonging to the *Cyperaceae*, especially in the *Caricoideae*. The genus comprises 75 species worldwide (Vánky 2002), distributed mainly in the Northern hemisphere.

The teliospores germinate with a characteristic two-celled basidium (Brefeld 1895) after an obligatory resting period in winter. The basidiospores germinate either by forming hyphae or secondary spores. No fusion between basidiospores or hyphae has been observed. Therefore, *Anthracoidea* is presumed to be (pseudo-)homothallic (Kukkonen & Raudaskoski 1964, Vánky 2002).

On the basis of extensive germination experiments (Lehtola 1940, Kukkonen 1961) a division of the genus *Anthracoidea* into the subgenera *Proceres* and *Anthracoidea* was proposed by Kukkonen (1963). These two subgenera were stated to differ mainly in the size and the nuclear behaviour of the basidiospores ('sporidia'; Lehtola 1940, Kukkonen 1963). In species of subgenus *Anthracoidea*, the basidium produces several small, globose to ovoid basidiospores per basidial cell. In contrast, the basidiospores of subgenus *Proceres* are very long and rod-shaped, with only one being produced per basidial cell. A further difference is found in the behaviour of the nuclei (Kukkonen & Raudaskoski 1964). In subgenus *Anthracoidea*, only one of the two nuclei of each basidial cell enters the basidiospore, whereas in subgenus *Proceres* both nuclei enter the young basidiospore. Unfortunately, the conditions for germination of the spores are difficult to reproduce *in vitro* and, consequently, the germination type of many species is still unknown.

In addition, the teliospore morphology in subgenus *Proceres* appears rather uniform. The teliospores are comparatively large, and nearly round to slightly irregular. The spore wall is evenly thickened, without internal swellings or light-refractive areas. The ornamentation is uniform, and neither completely smooth nor truly echinosporous forms are seen in this subgenus. In contrast, the spore morphology of species in subgenus *Anthracoidea* is heterogeneous, the shape varying from globose (e.g. *A. inclusa*) to extremely angular (e.g. *A. caricis*), and the spore ornamentation

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ranging from nearly smooth (e.g. *A. elynae*) to distinctly echinate (e.g. *A. subinclusa*). Because of these differences, Kukkonen (1963) divided subgenus *Anthracoidea* into three sections: species in sect. *Leiosporae* are characterized by evenly globose teliospores with a smooth surface; members of sect. *Anthracoidea* ('*Angulosporae*') have irregularly shaped spores with a moderately verrucose surface; and sect. *Echinosporae* for species with globose spores with an extremely verrucose to echinate surface.

At the species level, *Anthracoidea* species, like many other groups of the *Ustilaginales*, provide only few morphological characters that are taxonomically useful. The size and the wall structure of the teliospores are easily accessible, but reveal few distinct characters which are furthermore not exclusively ascribable to one single species. Therefore, the systematic position of the hosts is used in many cases to separate morphologically similar *Anthracoidea* species (e.g. Kukkonen 1963, Nannfeldt 1977, 1979, Vánky 1979, 1983).

The aim of this study was to elucidate the species concept and the evolution of the genus *Anthracoidea* by using molecular data.

MATERIALS AND METHODS

DNA extraction, PCR and sequencing

The organisms included in this study are listed in Table 1. Where possible, more than one specimen of each species was analyzed to estimate the genetic diversity within species and to prove the identity of the specimens from different localities.

DNA was isolated from the central parts of a single sorus using DNeasy™ Plant Mini kit (Qiagen, Hilden) according to the manufacturer's protocol. 1100 bp of the nuclear LSU rDNA were amplified by polymerase chain reaction (PCR) using the primers LR6 and LR0R (Moncalvo, Wang & Hseu 1995). In some cases, only the 5′-region of the LSU rDNA could be amplified using NL1 and NL4 as primers (Boekhout, Fell & O'Donnell 1995). PCR products were purified using the QIAquick™ purification kit (Qiagen) followed by an ethanol precipitation. Both strands were sequenced with the PerkinElmer ABI PRISM™ Dye-Termination Cycle Sequencing kit (Applied Biosystems, Weiterstadt) on automated sequencers (ABI 373A and ABI 3100, Applied Biosystems).

The sequences have been deposited in GenBank; and accession numbers are given in Table 1.

Data analysis

DNA sequences were aligned with POA (Lee, Grasso & Sharlow 2002); Lassmann & Sonnhammer (2002) provide a recent comparison of software packages for multiple sequence alignment. PAUP* version 4.0b10 (Swofford 2002) was used to perform neighbour-joining (NJ) and maximum parsimony analyses (MP) and the computer program MrBayes 3.0b3 (Huelsenbeck & Ronquist 2001) for Metropolis-coupled Markov chain Monte Carlo analyses (MCMCMC; Huelsenbeck *et al.* 2002).

Neighbour-joining analysis (Saitou & Nei 1987) was conducted with the Kimura-two-parameter distance model (Swofford *et al.* 1996). Support for internal nodes was estimated by 1000 neighbour joining bootstrap replicates (Felsenstein 1985) under the same model settings.

A heuristic search under the unweighted maximum parsimony criterion was conducted including 10 000 random sequence addition replicates with subsequent TBR branch swapping (MULTREES option in effect, STEEPEST option not in effect), each replicate being limited to 100 000 rearrangements. Gaps were treated as missing data. A second search strategy followed the parsimony ratchet approach (Nixon 1999) as implemented in PAUPRat (Sikes & Lewis 2001) using default values. No shorter trees than in the first approach could be obtained.

Support for the internal nodes of the trees was calculated by bootstrap analysis (Felsenstein 1985) using 1000 replicates. Every bootstrap replicate performed ten random sequence addition replicates with subsequent TBR branch swapping, each replicate being limited to 100 000 rearrangements.

For Bayesian analysis, four incrementally heated simultaneous MCMC Markov chains were run over 3 M generations using the general time reversible model (six rate classes) including a proportion of invariant sites and gamma distributed substitution rates of the remaining sites (GTR + I + G, see Swofford *et al.* 1996). Trees were sampled every 100th generation, resulting in an overall sampling of 30 000 trees. From these, the first 3000 trees were discarded as burn-in. MrBayes was used to compute a 50 % majority rule consensus of the remaining trees to obtain estimates for the *a posteriori* probabilities. This analysis was repeated seven times, always using random starting trees and default starting values to test the reproducibility of the results.

Microscopical studies

The teliospores of all organisms listed in Table 1 were studied using a light microscope (LM) with oil immersion. Dried spores were mounted and rehydrated in lactophenol (Savile 1987) to compare their sizes with the data given in literature and to determine the species (mainly according to Kukkonen 1963, Nannfeldt 1979, Vánky 1994).

RESULTS

The length of the alignment was 1221 bp. After exclusion of ambiguously aligned regions, 1079 bp including 396 variable and 280 informative sites remained for

Table 1. Species analyzed in this study

Species	Host	Locality/ Voucher[a]	GenBank accession no.
Anthracoidea arenaria	*Carex arenaria*	Germany, PUL F916	AY563606*[b]
A. aspera	*C. chordorrhiza*	Sweden, HMH 2774	AY563607
A. baldensis	*C. baldensis*	Switzerland, HMH 2861	AY563599
A. bigelowii	*C. bigelowii*	Sweden, HMH 2733	AY563566
	C. bigelowii	Russia, Kamchatka HMH 927	AY563567
	C. paupercula	Sweden, HMH 2736	AY563568
A. buxbaumii	*C. buxbaumii*	Sweden, HMH 2744	AY563582*
A. capillaris	*C. capillaris*	Sweden, HMH 2769	AY563596
A. caricis	*C. pilulifera*	France, HMH 2364	AY563589
A. caricis-albae	*C. alba*	Switzerland, HMH 2869	AY563594
	C. alba	Germany, HMH 2873	AY563595
A. carphae	*Carpha alpina*	Tasmania, M 40218	AY563614*
A. curvulae	*Carex curvula*	Switzerland, HMH 3912	AY563611
	C. curvula	France, HMH 2380	AY563612*
A. elynae	*Kobresia myosuroides*	Switzerland, HMH 3958	AY563609
	K. myosuroides	Germany, M 6794	AY563610*
A. globularis	*Carex globularis*	Finland, HMH 2422	AY563593
A. heterospora	*C. elata*	Finland, HMH 2438	AY563600
	C. elata	Russia, Kamchatka HMH 921	AY563601
A. hostianae	*C. hostiana*	Austria, HeRB 4706	AY563581*
A. inclusa	*C. rostrata*	Austria, HMH 2883	AY563605
A. irregularis	*C. digitata*	Slowenia, HMH 933	AY563592
	C. ornithopoda	Germany, HMH 3480	AY563590
	C. ornithopoda	Switzerland, HMH 3520	AY563591
A. karii	*C. brunnescens*	Sweden, HMH 2777	AY563575
	C. echinata	Norway, HMH 3676	AY563577
	C. echinata	Austria, HMH 3414	AY563578*
	C. echinata	Switzerland, HMH 3892	AY563576
	C. paniculata	Switzerland, HMH 3890	AY563574
	C. lachenalii	Sweden, HMH 2644	AY563579
A. '*karii* 2'	*C. davalliana*	Switzerland, HMH 3898	AY563608
A. lasiocarpae	*C. lasiocarpa*	Finland, HMH 972	AY563583*
A. limosa	*C. limosa*	Finland, HMH 2428	AY563572
	C. limosa	Sweden, HMH 2790	AY563573
A. misandrae	*C. atrofusca*	Sweden, HMH 2653	AY563584
A. paniceae	*C. panicea*	Switzerland, HMH 2818	AY563580
A. pratensis	*C. flacca*	Germany, HMH 1164	AY563564
	C. flacca	Austria, HMH 3599	AY563563
	C. flacca	Switzerland, HMH 3870	AY563565
A. rupestris	*C. rupestris*	Switzerland, HMH 3948	AY563598
A. '*rupestris* 2'	*C. glacialis*	Sweden, HMH 3692	AY563588
A. sclerotiformis	*Uncinia rubra*	New Zealand, M 4946	AY563613*
A. sempervirentis	*Carex ferruginea*	Germany, HMH 3616	AY563587
	C. firma	Germany, HMH 3612	AY563585
	C. sempervirens	Switzerland, HMH 3950	AY563586
A. subinclusa	*C. hirta*	France, HMH 3700	AY563604
	C. riparia	Germany PUL F915	AY563603*
	C. vesicaria	Germany, HMH 2809	AY563602
A. turfosa	*C. dioica*	Sweden, HMH 2797	AY563571
	C. parallela	Sweden, HMH 2523	AY563570
	C. heleonastes	Sweden, HMH 2662	AY563569
A. vankyi	*C. muricata* ssp. *muricata*	Switzerland, HMH 1305	AY563597
Gymnocintractia montagnei	*Rhynchospora alba*	MP 1838	AF009881*
Ustilago cynodontis	*Conodon dactylon*	MP 2344	AJ236150

[a] Collection numbers of: FO, F. Oberwinkler; HeRB, herbarium R. Berndt; HMH herbarium M. Hendrichs; MP, herbarium M. Piepenbring. Herbarium abbreviations: M, München; PUL, Kriebel Herbarium, Purdue, USA.

[b]*, Partial sequence obtained by using NL1–NL4 (Boeckhout *et al.* 1995) as primers.

analysis. The alignment is available upon request. All topologies were rooted with two species of the *Ustilaginales*, *Ustilago cynodontis* and *Gymnocintractia montagnei*.

For *Anthracoidea* specimens older than 2(−3) yr, the yield of extracted DNA decreased dramatically. This finding strikingly parallels the duration time given for isozyme activity (Salo & Sen 1993).

The result of the neighbour-joining (NJ) analysis is illustrated in Fig. 1. Maximum parsimony (MP) analysis revealed similar groupings. A 50% majority-rule consensus tree of the 201 most parsimonious

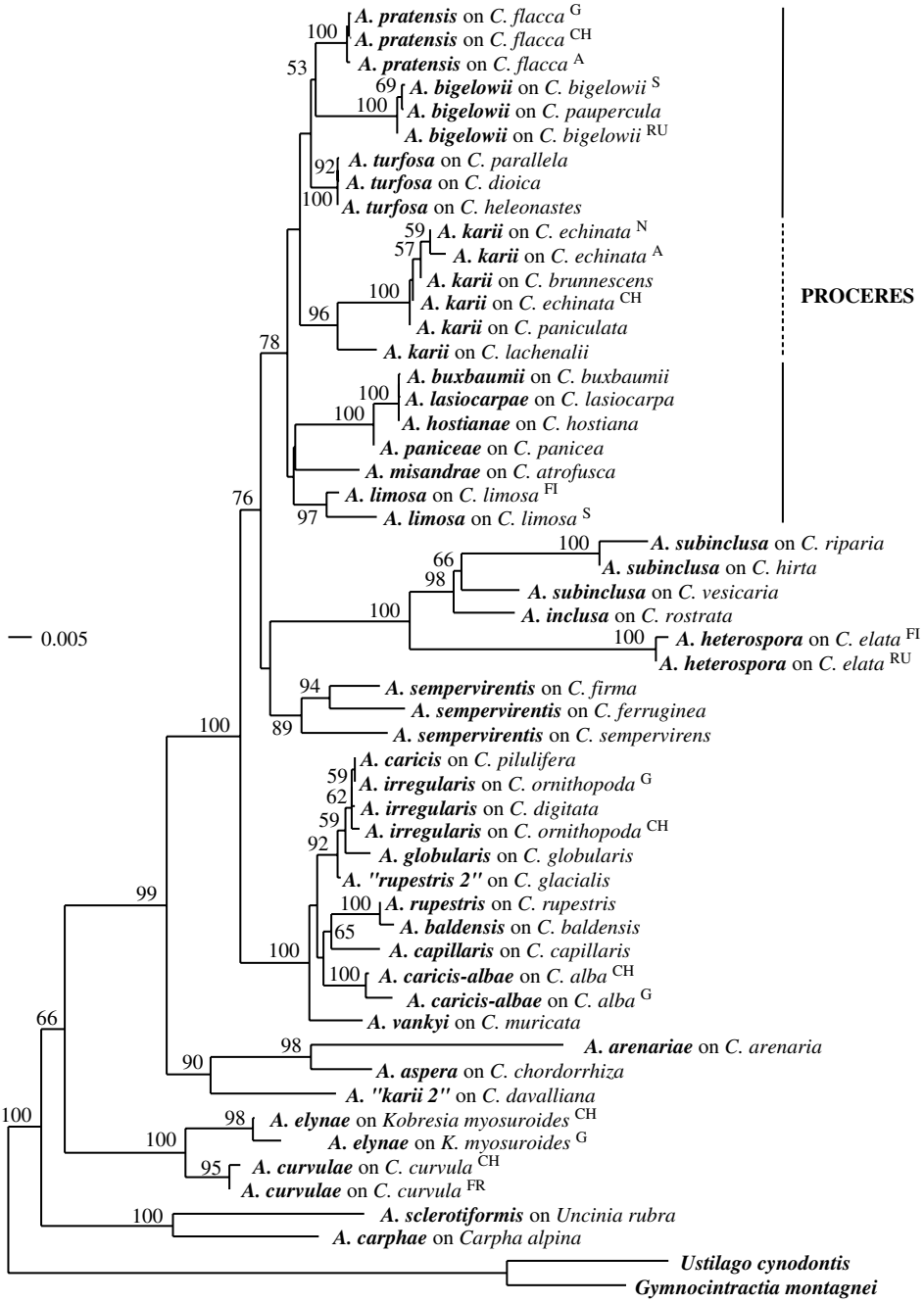

Fig. 1. Phylogram obtained by neighbour-joining analysis using the Kimura-2-parameter model of the nuclear LSU region sequences. The topology was rooted with *Ustilago cynodontis* and *Gymnocintractia montagnei*. Percentage bootstrap values of 1000 replicates are given, values smaller than 50 % are not shown. Branch lengths are scaled in terms of expected numbers of nucleotide substitutions per site. The different geographical sources of species are indicated by abbreviations for the respective countries (Table 1). Subgenus *Proceres* is labelled; for the species marked by a dotted line, examination of the teliospore germination is necessary (see p. 38).

trees obtained by our heuristic search strategy with parsimony ratchet approach (settings see above; Nixon 1999) is illustrated in Fig. 2. The consistency index of trees was 0.570 and the retention index 0.780 (Farris 1999).

The different runs of Bayesian Markov chain Monte Carlo (MCMC) analysis yielded consistent results. The topology of the 50 % majority-rule consensus was nearly the same as in the MP analysis and is shown in Fig. 2. In general, statistical support was lower in MP than in MCMC analysis. The tree topologies of the MP and MCMC analyses correlated with that of the NJ analysis in large parts, showing important differences only with respect to the circumscription of the subgenus *Proceres*. Thus, subgenus *Proceres* appeared monophyletic in neighbour-joining analysis with moderate support, but not in the MP and MCMC analyses (*cfr* Figs 1–2).

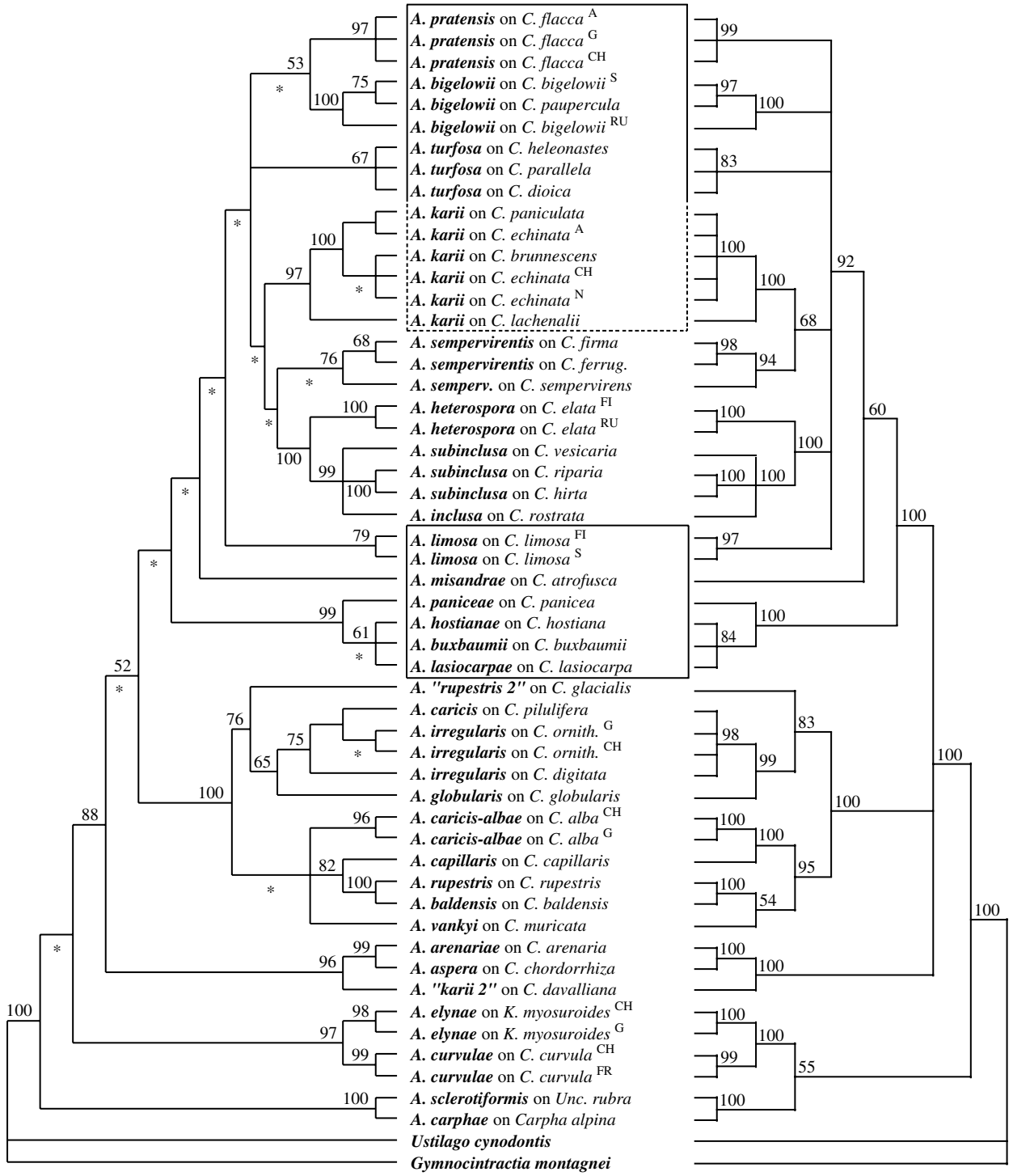

Fig. 2. *Left side* Maximum parsimony analysis of the nuclear LSU rDNA of *Anthracoidea* species. The topology was rooted with *Ustilago cynodontis* and *Gymnocintractia montagnei*. A majority-rule consensus tree of 186 best trees found in heuristic search is shown. Numbers on branches are bootstrap values from 1000 replicates. Branches marked with an asterisk under the line do not appear in the strict consensus tree. *Right side* Bayesian Markov chain Monte Carlo analysis of the same data set using the general time reversible model of DNA substitution with gamma distributed substitution rates and estimation of variant. Numbers on branches represent their respective *a posteriori* probabilities. Probability values below 50% are not shown. The different geographical sources of species are indicated by abbreviations for the respective countries (*cfr* Table 1). Species of subgenus *Proceres* are marked by a box; for the species surrounded by a dotted line, examination of the teliospore germination is necessary.

Subgenus *Anthracoidea* appeared paraphyletic in all three molecular trees. The subdivision of subgenus *Anthracoidea* into three sections (Kukkonen 1963) was questioned earlier (Nannfeldt 1977), and was not fully supported by our molecular results. Thus, section *Echinosporae* appeared as monophyletic when

A. aspera was not included. Section *Leiosporae* including *A. curvulae* could be interpreted as monophyletic. Section *Anthracoidea* (*Angulosporae*) was subdivided into at least four subgroups, but a group of species with a very irregular to angular spore shape was highly supported as a monophyletic lineage in all analyses. The species concept and the wide distribution range of some species was supported in most cases, and only in some does any revision seem to be required.

DISCUSSION

In contrast to observations in other smut genera (Begerow, Lutz & Oberwinkler 2002, Stoll *et al.* 2003) and the extreme length variation of the ITS rDNA (Juuti & Salo 2002), the LSU rDNA region proved highly suitable for phylogenetic investigations within the genus *Anthracoidea*.

Well supported groups and species concept

As in other genera of the *Ustilaginales*, the first attempts to classify *Anthracoidea* species were mostly based on the morphology of the teliospore (Brefeld 1895, 1912, Clinton 1904, 1906, Sydow 1924, Ciferri 1931, Liro 1938). With the increase of successful germination experiments (Lehtola 1940, Kukkonen 1961, 1963, Boidol & Poelt 1963) more characters became available. Additional information was obtained from the phylogeny of the host genus (Savile 1951, 1952, Nannfeldt 1977, Nannfeldt & Lindeberg 1957), i.e. for *Anthracoidea* the genera *Carex*, *Carpha*, *Fuirena*, *Kobresia*, *Schoenus*, *Trichophorum* and *Uncinia* of the *Cyperaceae* (Vánky 2002). In recent studies, a narrow host species concept has been favoured for the genus *Anthracoidea* (Nannfeldt 1979, Vánky 1985, 1994), neglecting the problem that some taxa now share very similar morphology (*cfr* Vánky 1985). In this study, for the first time sequence data are used to assess the species circumscription of the genus *Anthracoidea*. The discussion of these species mainly follows their position in the NJ tree (Fig. 1) from the base to the top.

Anthracoidea sclerotiformis, parasitizing *Uncinia* spp. from South America to Australia, clustered together with *A. carphae*, described on *Carpha alpina* from southern Argentina and New Zealand, with 100% support in all analyses. Both species share similar teliospore morphology with medium-sized and irregular to angular spores bearing fine ornamentation (Cunningham 1924, Kukkonen 1963, Zambettakis 1978, Vánky 1979). Therefore, Kukkonen (1963) integrated *A. sclerotiformis* into section *Anthracoidea*, although the germination type is unknown for both species. *Uncinia* was interpreted as a possible ancestor of the genus *Carex* (e.g. Kükenthal 1909, Kreczetovicz 1936, Nelmes 1952), which might be supported by the position of *A. sclerotiformis* in our molecular trees. The recent investigations in the phylogeny of the genus *Uncinia* (Starr, Harris & Simpson 2002, 2003) may stimulate further studies in the *Anthracoidea* species parasitizing this interesting host genus.

In all our analyses, *A. elynae* and *A. curvulae* formed a well-supported cluster. These species are distinguished morphologically from all others in our analyses by the globose and nearly smooth teliospores, which are typical of members of the section *Leiosporae*. The difficulties in the circumscription of section *Leiosporae* as having globose spores with evenly thin walls and a more or less smooth surface have already been discussed by Nannfeldt (1977), and is reinforced by the molecular results: Since no species of *Anthracoidea* described so far has an absolutely smooth spore surface, a gradation of this character was assumed by Nannfeldt (1977). Thus, *Anthracoidea curvulae* normally shows fine warts on the spore surface and was therefore considered to be a member of section *Anthracoidea*. Taking into account that some LM preparations of *A. curvulae* contained nearly smooth spores, we consider that section *Leiosporae* also includes *A. curvulae*. An additional morphological trait for section *Leiosporae* is the comparatively large basidiospores ('sporidia'; Kukkonen 1963, Nannfeldt 1977). Unfortunately, our germination experiments failed, and therefore the germination type and the size of the basidiospores of *A. curvulae* remain unknown.

A. elynae parasitizes *Kobresia myosuroides* in most parts of its wide distributional range in the Northern Hemisphere. We included two specimens from the German and Swiss Alps in the analysis, but a wider species sampling is needed to verify the homogeneity of this species. The specimens of *A. curvulae*, collected on *C. curvula* in the Swiss and French parts of the central Alps, shared identical sequences; the slight difference in branch length in NJ tree was based on different sequence lengths (*cfr* Table 1). The systematic position of *C. curvula* has been controversial since Kükenthal's (1909) classification within the subgenus *Vignea* (e.g. Ivanova 1939, Chater 1980). Ivanova (1939) treated *C. curvula* within a larger defined genus *Kobresia*. The well-supported relation of *A. curvulae* to *A. elynae* in all analyses gives support for the later interpretation.

A third cluster in the molecular tree is well supported (90% NJ bootstrap, 96% MP bootstrap, 100% *a posteriori* probability), and comprises species with different spore morphologies. However, all three species parasitize members of *carex* subgenus *Vignea*. In addition, at least *A. aspera* and *A. arenariae* have the same germination type as subgenus *Anthracoidea*. The distinct warts of *A. aspera* differ from the spines of 'true' echinosporous species mainly by their moderate height (max. 1 μm and rounded tips. Consequently, the spore ornamentation of *A. aspera* can be regarded as an extreme form in section *Anthracoideae* (Nannfeldt 1977, 1979), but distinct from the ornamentation of

species in section *Echinosporae* (see below), where *A. aspera* has previously been placed (Kukkonen 1963). The Swiss *Anthracoidea* specimen on *C. davalliana* has small and subglobose spores with a nearly smooth surface. It is morphologically identical to *A. karii*, which is also known to parasitize *C. davalliana*. Since the host species for the type of *A. karii* is *C. brunnescens* (see below), we provisionally refer to this specimen as '*A. karii* 2'. The wide host range of *A. karii* was critically noted by Nannfeldt (1979), but the morphological differences were not sufficient to separate species. Our studies indicate that a morphologically similar species occurs on *C. davalliana*, which can be separated by molecular data. The problem of germination type and the question of the correct ascription of *A. karii* to subgenus *Anthracoidea* will be discussed below.

The fourth group includes the type species of the genus *A. caricis*, and is optimally supported in all analyses (100 %). Species included here are well defined by their extremely irregular to angular teliospores with small size variations in the range of (19–)20–22(−23) × 16(−18) μm and finely verrucose ornamentation. The spore wall is unevenly thickened, normally 1.5–3(−4) μm, but sometimes up to 7 μm thick. The basidia are of the *Anthracoidea* type (Brefeld 1895, 1912, Lehtola 1940, Boidol & Poelt 1963, Kukkonen 1963). Interestingly, the host range covers all three subgenera of *Carex*. Because this group contains the type-species of *Anthracoidea*, it must be referred to as sect. *Anthracoidea* (*syn. Angulosporae*).

A. rupestris is considered to parasitize both *C. rupestris* and *C. glacialis* (*cfr* Lehtola 1940, Kukkonen 1963, Vánky 1994). However, our data support a separation of the two smuts on the different hosts at the species level. A small difference in the size of the basidiospores ('sporidia') of the *Anthracoidea* species on *C. rupestris* and *C. glacialis* was noted by Kukkonen (1963). Nannfeldt (1979) pointed out the problem of the discrete systematic position of the host species. *Carex rupestris* is classified in subgenus *Primocarex* (Kükenthal 1909, Chater 1980, Ball 2002). The controversial systematic position of *C. glacialis* was discussed (Savile & Calder 1953, Chater 1980); Ball & Murray (2002) proposed a close relationship to *C. rupestris*.

A. baldensis is revealed as the closest relative of *A. rupestris* on *C. rupestris* in all analyses. *C. baldensis* is traditionally ascribed to subgenus *Vignea* (*cfr* Kükenthal 1909, Chater 1980). *Anthracoidea vankyi* is a second species in this group parasitizing a member of subgenus *Vignea* (*C. muricata*).

All other species in this angulosporous cluster parasitize *Carex* species of different sections within subgenus *Eucarex*. In general, the topology within this well-defined group remains unresolved, and the species concept is unclear in some parts. Thus, the morphological characters of *A. irregularis* and *A. caricis* are similar and the (re-)integration of *A. irregularis* in *A. caricis*, which was first proposed by Kukkonen (1963), seems to be supported by LSU sequence data. Possibly, *A. caricis* parasitizes different hosts, whereas species like *A. globularis*, *A. capillaris* and *A. caricis-albae* are restricted to one host species each. Sydow's (1924) assumption that *A. caricis-albae* is closely related to the *Anthracoidea* species on *C. digitata* and *C. ornithopoda* was doubted first by Liro (1938) and is also in contrast to our molecular results. A detailed investigation of this group covering additional species (e.g. *A. pseudoirregularis*, *A. michelii*, *A. tomentosae*) and using a more variable DNA region may contribute to the question of species delimitation. Moreover, the recent investigations especially on the genus *Carex* (Starr, Bayer & Ford 1999, Roalson, Columbus & Friar 2001, Roalson & Friar 2004, Hendrichs *et al.* 2004a, b) may stimulate these studies by providing new phylogenetic data from the hosts.

The fifth group in the NJ tree is nested within subgenus *Proceres* in MP and MCMC analyses. The sister relationship of *A. sempervirentis* to the highly supported group comprising *A. heterospora*, *A. inclusa* and *A. subinclusa* was not supported in our analyses. For all these species germination experiments revealed a basidium of the *Anthracoidea* type (Brefeld 1912, Lehtola 1940, Kukkonen 1963, Boidol & Poelt 1963, Ingold 1989). The high support (100 % in all analyses) for a grouping of the echinosporous species *A. inclusa* and *A. subinclusa* together with *A. heterospora* with nearly smooth teliospores is surprising. *A. heterospora* was found several times on *C. elata*, and the two specimens from Finland and Kamchatka included in our analyses differed in only one bp on the total length of 1012 bp; this wide distribution range was postulated earlier (Nannfeldt & Lindeberg 1965, Nannfeldt 1979, Vánky 1994). The echinosporous species of *Anthracoidea* have been intensively studied (Lehtola 1940, Kukkonen 1964, 1969, Salo & Sen 1993, Ingvarsson & Ericson 1998, 2000). The characterization of species by spore morphology and the dimensions and spacing of the spines or papillae has resulted in different delimitations since the early investigations of mostly European specimens (*cfr* Rabenhorst 1874, Sydow 1924, Liro 1938, Lehtola 1940). Adding North American specimens to the European species (Clinton 1906, Savile 1951, 1952), the picture became more complicated and numerous transitional stages between the 'pure' species have been found. Nannfeldt (1979) named a species *A. intercedens* to indicate the intermediate morphology. He finally differentiated the echinosporous species by the ultrastructure of the ornaments of the spines and the spore surface between the spikes as seen in SEM (Nannfeldt 1979). Our molecular data support the assumption that species delimitations are vague in this group. Thus, *A. subinclusa* on *C. vesicaria* appears to be different from *A. subinclusa* found on *C. hirta* and the type host *C. riparia*. As for *A. subinclusa*, the host range is also far from fixed for most species in this group.

The sixth group in the NJ tree comprises species which ascribed to subgenus *Proceres* (see p. below). The position of *A. misandrae* within subgenus *Proceres* is not clearly fixed and changes in different analyses (*cfr* Figs 1–2).

The spore size of *A. limosa* is very variable, and especially on *C. rariflora* and its hybrids, very large-spored specimens are found (Lehtola 1940, Savile 1952), whereas the teliospores of *A. paniceae*, *A. lasiocarpae*, *A. buxbaumii* and *A. hostianae* have a similar size, form, and surface structure. These last species are morphologically inseparable without additional information on the host species. The nearly identical LSU sequences of *A. hostianae*, *A. lasiocarpae* and *A. buxbaumii*, the identical teliospore morphology and germination type, and the coexistence of the host species at many locations, make the separation of these species questionable.

For *A. karii*, germination of the subgenus *Anthracoidea* type is traditionally presumed. Unfortunately, the germination of *A. karii* on the type host *C. brunnescens* was never studied. Lehtola (1940) described germination of the *Anthracoidea* type for *A. karii* on *C. dioica*. Therefore, it appears necessary to examine the germination of *A. karii* on the type host. Unfortunately, our germination experiments failed repeatedly. Consequently, we labelled this group with a dotted line in the *Proceres* cluster in our molecular trees.

Our molecular results separate *A. karii* on *C. brunnescens* in the *Proceres*-group clearly from the parasite on *C. davalliana* ('*A. karii 2*'), for which germination of the *Anthracoidea* type has to be assumed. For a correct circumscription of this species on *C. davalliana*, the small-spored species described on the closely related host *C. dioica* (Lehtola 1940) will also have to be studied in detail.

A weak infection of *C. paniculata* turned out to be due to *A. karii*, which parasitized *C. echinata* nearby. Accordingly, *C. paniculata* has to be regarded as an additional host for *A. karii* (*cfr* Tulasne & Tulasne 1847). Like *A. karii*, the large-spored *A. turfosa* exclusively parasitizes species of subgenus *Vignea* and is frequently found on hosts ascribed to section *Dioicae* and nearly all possible hybrids in northern Europe. For *A. bigelowii*, the wide distribution on *C. bigelowii* was confirmed by the comparison of LSU sequences of specimens from Sweden and from Kamchatka. In Swedish Lappland a population of *C. paupercula*, which grew in the vicinity of heavily infected *C. bigelowii* species, also carried a weak infection of *A. bigelowii*, making it an accidental host (*cfr* Lehtola 1940). The homogeneity of *A. pratensis* throughout Europe was presumed by several authors (e.g. Ciferri 1931, Kochman 1934, Liro 1938, Lehtola 1940, Boidol & Poelt 1963), and is supported by sequence data of specimens collected from three different localities in Central Europe.

Subdivision of the genus

The molecular data give no clear result regarding the monophyly of subgenus *Proceres*. The interpretation as a monophyletic group according to NJ analysis is only moderately supported. In addition, members of subgenus *Anthracoidea*, including *A. subinclusa*, *A. inclusa*, *A. heterospora* and *A. sempervirentis*, nested within species of subgenus *Proceres* in the consensus trees obtained by MP and MCMC analysis, respectively. Our analyses neither support nor significantly reject the hypothesis for a subdivision of the genus *Anthracoidea* into two subgenera. However, the interpretation of the subgenus *Proceres* as a phylogenetically old lineage (e.g. Kukkonen 1963, Zambettakis 1978) was not supported by our molecular results. The totally different germination behaviour combined with a uniform spore morphology of subgenus *Proceres* may possibly have occurred only once and late in the evolution of the genus *Anthracoidea*.

Subgenus *Anthracoidea* and section *Anthracoidea* have been revealed as paraphyletic in all three analyses (*cfr* Kukkonen 1963, 1969). Thus, the representatives of subgenus *Anthracoidea*, which are grouped in a sister position to (NJ) or nested within (MP, MCMC) species of the *Proceres* group, include members of sections *Anthracoidea* (*A. sempervirentis*, *A. heterospora*) and *Echinosporae* (*A. inclusa*, *A. subinclusa*). The species ascribed to section *Leiosporae* (*A. elynae*, *A. curvulae*) are nested among members of section *Anthracoidea* (e.g. *A. sclerotiformis*, *A. arenariae*).

However, there was a highly supported (100 % in all analyses) core-group comprising species of section *Anthracoidea* having extremely irregular spores and an unevenly thickened spore wall (i.e. *A. caricis*, *A. irregularis*, *A. gobularis*, *A. rupestris*, *A. baldensis*, *A. capillaris*, *A. caricis-albae*, and *A. vankyi*). This group included the type species, *A. caricis* on *C. pilulifera*, and it seems appropriate to treat it as section *Anthracoidea* (i.e. '*Angulosporae*') in the strict sense. In addition, the two representatives of section *Leiosporae* (*A. elynae*, *A. curvulae*) as well as the two representatives of section *Echinosporae* (*A. subinclusa* and *A. inclusa*) clustered together with high support.

The phylogenetic significance of the sections in the subgenus *Anthracoidea* was doubted earlier (Nannfeldt 1977), which is corroborated by the molecular results. Thus, the traditional ascriptions can cover only a part of the diversity of species within the subgenus *Anthracoidea*.

Coevolution

On the basis of the limited species sampling only a few deductions in respect of the question of coevolution with the host plants can be drawn so far. The species parasitizing species of *Carpha* and *Uncinia*, which are considered to be ancestral to the genus *Carex* by some authors (e.g. Kükenthal 1909, Kreczetovicz 1936,

Nelmes 1952), appeared in a sister group to the other *Anthracoidea* species, which exclusively parasitize species of *Carex* and the very closely related genus *Kobresia*. The close relationship of *A. elynae* and *A. curvulae*, as revealed by our data, supports the assumption of a closer relation of *C. curvula* to the genus *Kobresia*, which was first postulated by Ivanova (1939).

The colonization of species within subgenus *Vignea* has occurred at least three times, with one group in a sister position to the parasites on species of subgenus *Carex*, which is in accordance with the sister position of subgenus *Vignea* to the subgenus *Carex* (Kükenthal 1909, Yen & Olmstead 2000, Ball & Reznicek 2002). In contrast, our data indicate that the colonization of hosts within *Carex* subgenus *Carex* may have occurred only once and presumably late in the evolution of the genus *Anthracoidea*.

The recent progress in the understanding of the phylogeny of the host genera (Starr *et al.* 2002, 2003, Roalson *et al.* 2001, Roalson & Friar 2004, Hendrichs *et al.* 2004a, b) may stimulate further investigations to elucidate the joint evolution of the genus *Anthracoidea* and its hosts. Finally, the present paper and other publications (Lehtola 1940, Nannfeldt 1979, Vánky 1985, 1994) have reported the occurrence of accidental infections of different *Carex* spp. by *Anthracoidea* spp. not normally parasitizing them. These findings may have important implications for evolutionary trends within the genus *Anthracoidea*.

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