Short Communication

Evolution of the gall-forming plant parasitic nematodes (Tylenchida: Anguinidae) and their relationships with hosts as inferred from Internal Transcribed Spacer sequences of nuclear ribosomal DNA

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Abstract

Phylogenetic relationships among gall-forming plant parasitic nematodes of the subfamily Anguininae are reconstructed by maximum parsimony and maximum likelihood analyses. Sequences of the ITS of rDNA from 53 populations and species of gall-forming nematodes and five populations of the *Ditylenchus dipsaci* species complex were analysed. The phylogenetic trees strongly support monophyly of the genus *Anguina* and show nonmonophyly for the genera *Mesoanguina* and *Heteroanguina*. Morphological and biological characters are generally congruent with the anguinid groups identified in the rDNA phylogeny. Analyses of evolution of different gall types among anguinids reveal that there are apparent evolutionary trends in gall evolution: from abnormal swelling and growth of infested plant organs toward small localised galls, and from infestation of vegetative toward generative organs. Our study demonstrates that the main anguinid groups are generally associated with host plants belonging to the same or related systematic groups. The comparison of the ITS phylogenies of anguinids parasitising Poaceae and their host grasses shows a high level of cospeciation events.
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1. Introduction

The gall-forming nematodes of the subfamily Anguininae are obligate specialised parasites of plants on which they induce galls in various organs. Over 40 nominal species of gall-forming nematodes have been described. Several anguinid species (*Anguina tritici*, *A. agrostis*, *A. funesta*) are considered of economic importance as agricultural and quarantine pests in various countries. (Chizhov and Subbotin, 1990; Krall, 1991).

Two classifications of the gall-forming nematodes with different genus composition have been proposed by Brzeski (1981) and Chizhov and Subbotin (1990). The phylogeny of anguinids has been studied at the generic level based on morphological and biological characteristics. However, these data failed to yield well supported relationships (Chizhov and Subbotin, 1990). The alternative biochemical and molecular approaches appear more promising for phylogenetic analysis. Genomic relationships among some anguinids infesting grasses have been established on the basis of allozymes (Riley et al., 1988) and the ITS1 sequences of rDNA (Powers et al., 2001). However, these investigations involved only a limited number of species, and thus the general phylogeny of Anguininae remains unclear.

Consequently, the evolution of Anguinidae and the problems of the generic and species differentiation requires further study.

The phenomenon of galling has evolved independently many times in various organisms. The multiple origins of galling make it possible to search for convergent features in these independent evolutionary histories. Evolutionary trends in the process of formation of anguinid galls have been proposed by Paramonov (1970), Solovyeva and Krall (1983), and Chizhov and Subbotin (1990), but they have not been rigorously tested with phylogenetic methods.

Cospeciation occurs in many symbiotic associations as identified by analysis of molecular phylogenies: gopher and lice, fungal symbionts and grasses, chemoautotrophic bacteria and deep sea clams, bacterial symbionts and marine red algae, symbiotic bacteria and insects, pollinating wasps and figs (Clark et al., 2000; Lopez-Vaamonde et al., 2001). However, cospeciation is often obscured by host switching, or jumping, which appears to be particularly common in some associations (Roy, 2001). No studies exist that combine analyses of molecular phylogeny of parasitic nematodes and their host plants, and such studies would provide new contributions for better understanding plant-parasitic associations.

The present study includes a large sampling of taxa from the Anguinidae and evaluates whether ITS sequence variation can reveal taxonomic and phylogenetic relationships within gall-forming nematodes of this family using the maximum parsimony and maximum likelihood phylogenetic procedures. The principal objectives of the study were: (1) to compare the ITS-derived phylogeny with the current taxonomy, systematics and hypotheses of evolution and its congruency with the morphological and biological characters; (2) to ascertain the monophyly of the genera and their position within the subfamily Anguininae; (3) to estimate the species boundaries of the stem nematode *Ditylenchus dipsaci* and the bentgrass nematode *A. agrostis*; (4) to estimate constraints in gall evolution in anguinids; and (5) to compare molecular phylogenies of anguinids infesting grasses and their corresponding hosts and to estimate the level of their cospeciation.

2. Materials and methods

2.1. DNA isolation, PCR amplification, and sequencing

A total of 58 nematode samples were included in the study (first table of Appendix A). Identification of nematodes in this work has been made based on combined analyses of morphological, biological and molecular data. DNA extraction from nematodes using proteinase K and PCR amplification were performed as described by Zheng et al. (2000). Total plant DNA was extracted from dried tissues using DNeasy Plant Mini Kit (Qiagen, Germany). Two sets of nematode primers were used in the PCR reactions to amplify the 18S gene in part, ITS1, 5.8S gene, ITS2, and 28S gene in part: (1) TW81 and AB28 (Zheng et al., 2000) or (2) rDNA1 (5′-TTGATTACGTCCCTGCCCTTT-3′) and rDNA2 (5′-TTTCACTCGCCGTTACTAAGG-3′). The ITS region from plant material was PCR amplified with primers ITS5m and ITS4 (White et al., 1990). DNA fragments were directly sequenced in both directions with nematode TW81, AB28, 5.8SM2, or 5.8SM5 primers (Zheng et al., 2000) or plant ITS3 and ITS2 primers (White et al., 1990) using BigDye Terminator Cycle Sequencing Ready Reaction Kit (PE Applied Biosystems). Sequences were run on a 377 DNA sequencer (PE Applied Biosystems, UK).

2.2. Sequence alignments

The DNA sequences were aligned using ClustalX 1.64 with default options and then optimized manually. As sequences of two populations of *A. australis*, *A. tritici*, *Subanguina radicicola*, three populations of *Anguina funesta*, and four populations of *A. agrostis* were identical, only one sequence from each of these species was used for analyses. Several alignments were generated: (i) entire nematode ITS1 + 5.8S + ITS2 alignment for analyses of nematode phylogeny, (ii) nematode ITS1 + 5.8S + ITS2 alignment with reduced number of species, and (iii) plant ITS1 + ITS2 alignment for co-phylogenetic analysis. The ITS1 and ITS2 sequences for several grasses were obtained from the GenBank. All original nematode (AF396311–AF396368) and plant (AF498394–AF498396) sequences reported here have been deposited in the GenBank.

2.3. Phylogenetic analysis

Maximum parsimony (MP) and maximum likelihood (ML) analyses were conducted with PAUP*4b (Swofford, 2002). *Ditylenchus* species (Paramonov, 1970; Powers et al., 2001) and *Carex acuta* were used as outgroups for the construction of the nematode and grass phylogenies, respectively.

The MP analyses of the ITS alignments were performed by a heuristic search procedure with 100 replicates of random taxon addition. Gaps were coded in two ways, either as missing data or as a fifth character. For ML analysis, the appropriate substitution model of DNA evolution that best fitted the data set was determined using ModelTest 3.04 (Posada and Crandall, 1998). Bootstraps with 200 and 100 replicates were conducted for MP and ML analyses, respectively. The tests of alternative topologies were conducted by the ML methods of Kishino–Hasegawa and

Shimodaira–Hasegawa as implemented in PAUP*. The partition homogeneity test as implemented in PAUP* was used, and was run with 1000 replicates to determine whether the nematode phylogeny reflected plant phylogeny. Cospeciation events in nematodes and plant phylogeny were estimated using TreeMap1.0 (Page, 1994). The probability of obtaining the observed number of cospeciation events was calculated by randomisation of nematode and plants trees 1000 times with the proportional-to-distinguishable model to generate a null frequency distribution.

3. Results and discussion

3.1. Phylogenetic relationships within Anguinidae: congruence between ITS-based phylogeny and traditional classifications

Unweighted MP analysis with gaps coded as missing data, revealed 14 equally most parsimonious trees (Fig. 1). When gaps were coded as a fifth character, the MP analysis yielded four most parsimonious trees (tree length = 1257). A comparison of the topologies and
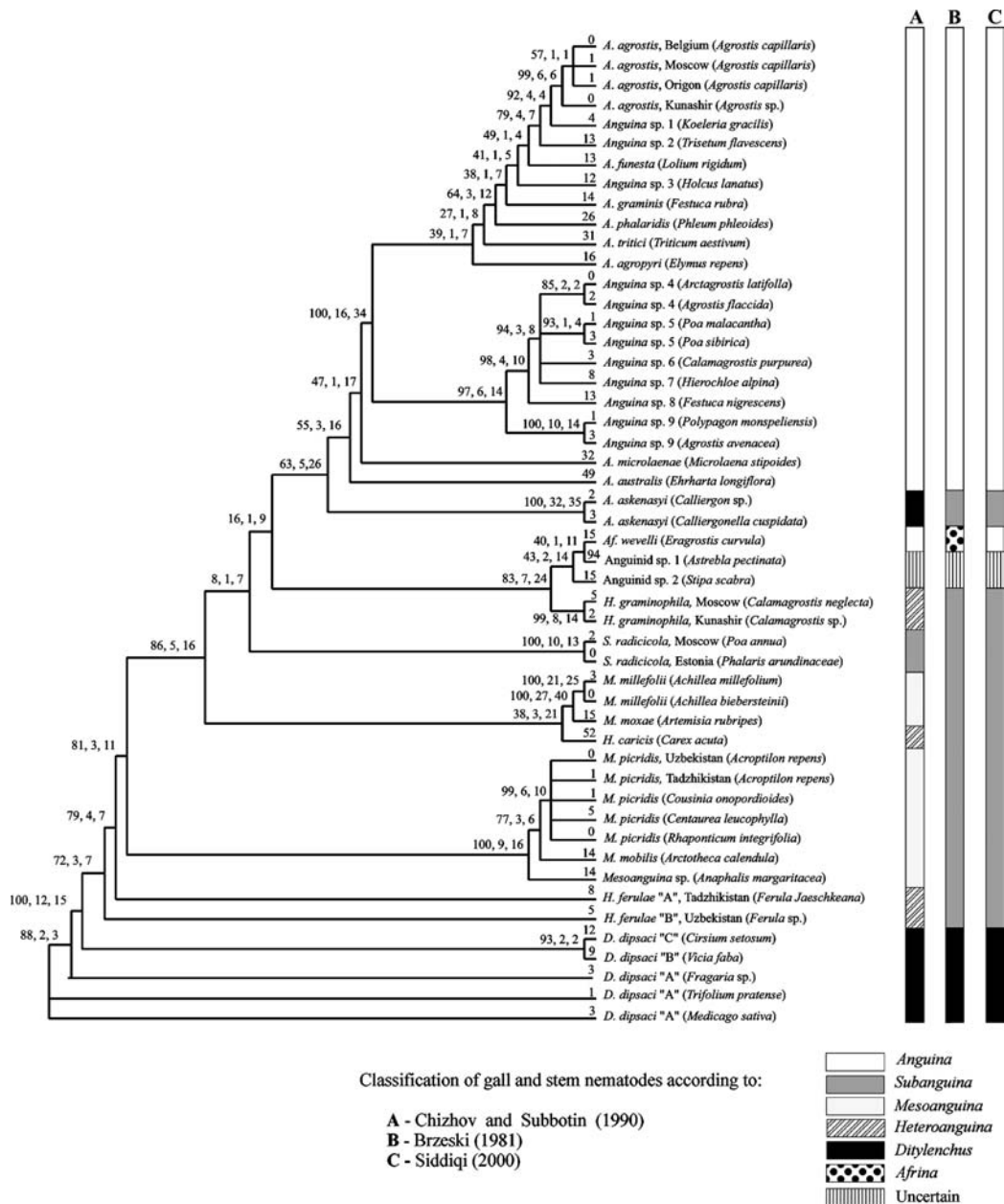

Fig. 1. Strict consensus of 14 equally parsimonious trees from analyses (unweighted parsimony analyses with gaps treated as missing data) of ITS sequence data for 50 species and populations of Anguinidae (tree length = 1046). Number above the branches indicates bootstrap percentage, decay index and number of character state changes.

bootstrap supports of clades obtained after both these procedures did not reveal any significant differences. MP trees were not significantly worse than the optimal ML tree (second table of Appendix A) and some differences in topology were corresponded to the weakest nodes (Fig. 2).

Paramonov (1970) stated that *Anguina* and *Ditylenchus* are phyletically close, and that *Anguina* is a very specialized branch arising from ancestral dylenchids. It was suggested that species of the genus *Heteroanguina* originated from ancestors common to contemporary ditylenchids, and occupy an intermediate position between plant pathogenic ditylenchids and anguinids (Chizhov and Subbotin, 1990). The genus *Heteroanguina* is polyphyletic with three distinct unrelated lineages branching at different locations in the phylogenetic trees (Figs. 1 and 2). The test of significance in likelihood differences rejects the monophyly of *Heteroanguina*, and the monophyly of species of this genus parasitizing monocots (second table of Appendix A). Thus, this genus can be considered a heterogenetic group of unrelated species with plesiomorphic state characters and taxonomic revision of this genus appears necessary.
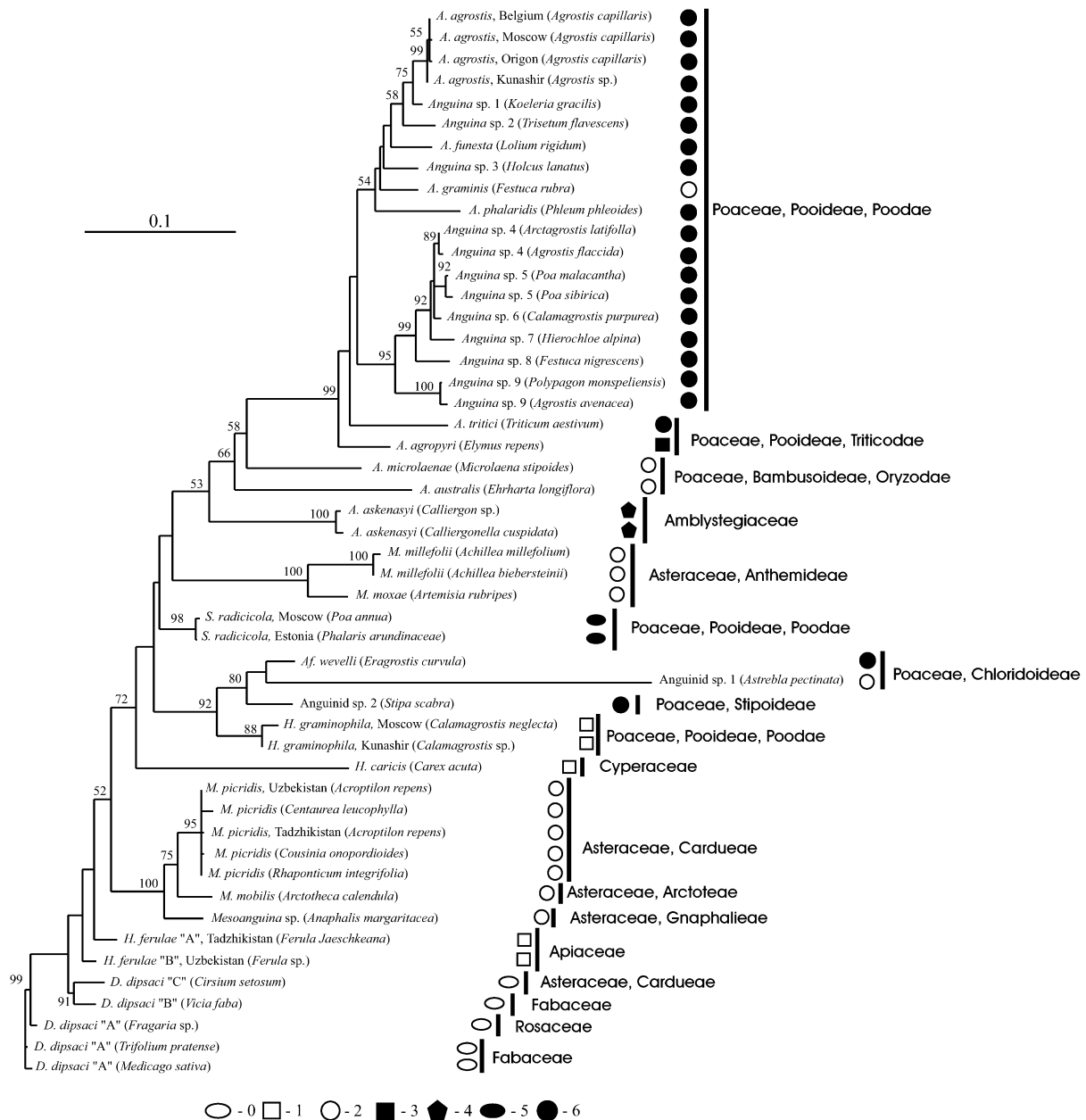

Fig. 2. The single maximum likelihood tree from analyses of ITS sequence data for 50 species and populations of Anguinidae obtained by PAUP* using with GTR + Γ + I model of DNA sequence evolution (Ln likelihood = −6269.51184) with mapping systematic position of host-plants and gall type. 0, abnormal growth and swollen; 1, large or elongated gall; 2, leaf and stem gall; 3, basal stem gall; 4, terminal stem gall; 5, root gall; 6, seed gall.

Solovyeva and Krall (1983) hypothesized that *H. caricis* had an ancient origin, possibly being derived from an anguinid branch infesting Poaceae. The position of *H. caricis* in our trees was not well resolved. However, the ITS data confirm their early diverging position among Anguininae. Solovyeva and Krall's (1983) hypothesis on the monophyletic origin of anguinids parasitizing grasses and sedges cannot be rejected (second table of Appendix A).

The molecular data indicate paraphyly of the genus *Mesoanguina* parasitizing Asteraceae (second table of Appendix A). The studied species of this genus cluster into two distinct groups in agreement with their peculiarities of morphology and host plant range.

Paramonov (1967, 1970) proposed the genus *Subanguina*, with *S. radicicola* as type species, and stated that it retained the ancestral type of ontogeny. *S. radicicola* is the only species of the Anguinidae that forms galls on roots of many grass species. The basal position of *S. radicicola* within Anguininae infesting grasses in the phylogenetic trees supports Paramonov's (1970) and Solovyeva and Krall's (1983) hypothesis of an ancient origin of this species. Based on similarity in structure of the preuteral glands, Brzeski (1990) transferred seventeen species to this genus. However, the ITS data (Fig. 1) support the narrow concept of *Subanguina* by Paramonov (1970) and Chizhov and Subbotin (1990) and not the broad concept proposed by Brzeski (1990).

The genus *Anguina* is monophylic. Different authors have placed *A. askenasyi* either in *Ditylenchus* (Chizhov and Subbotin, 1990; Kirjanova and Krall, 1971; Krall, 1991) or in *Subanguina* (Brzeski, 1981; Siddiqi, 2000). Our study does not support the inclusion of this species in the genus *Ditylenchus*; the test of the phylogenetic position of *A. askenasyi* rejected its relationships with *D. dipsaci* (second table of Appendix A). Two species, *A. australis* and *A. microlaenae* inducing leaf and stem galls in plants of the subfamily Bambusoideae occupy a basal position within *Anguina* species parasitizing Poaceae. Other species of this genus, mainly those inducing seed galls, form a highly supported clade (100%).

3.2. Species boundaries for stem and gall-forming nematodes

According to present understanding, *D. dipsaci* should be considered as a species complex. *D. dipsaci* consists of many races and populations that differ mainly in host range and appear to be at different stages of speciation and partial or complete reproductive isolation (Sturhan and Brzeski, 1991). Most populations from cultivated plants (*Fragaria* sp., *Medicago sativa*, *Trifolium pratense*) have $2n = 24$ chromosomes. The presence of several nucleotide autapomorphies for the giant race from *Vicia faba* ($2n = 48$–60), and a population from *Cirsium setosum* ($2n = 52$) and as well as the

high level of their sequence dissimilarities from *D. dipsaci sensu stricto* support the suggestion by Sturhan and Brzeski (1991) that probably these isolates have deserve species status.

Anguina agrostis and *A. phalaridis* were first recorded from seed galls of *Agrostis capillaris* and *Phleum phleoides*, respectively. There are numerous reports of galls found in various species and genera of grasses, but without satisfactory diagnostic descriptions of the associated nematodes. The grasses *Apera*, *Arctagrostis*, *Calamagrostis*, *Dactylis*, *Eragrostis*, *Festuca*, *Hordeum*, *Koeleria*, *Lolium*, *Phalaris*, *Phleum*, *Poa*, *Puccinellia*, *Sporobolus*, and *Trisetum* have been included in the list of host plants for *A. agrostis* (Kirjanova and Krall, 1971). Southey (1973), after reviewing literature on the host specificity of seed-gall nematode populations and their morphological and morphometrical differences from the type host, suggested that a thorough revision of species causing galls in flowers of grasses was required. He also suggested that *A. agrostis sensu stricto* appeared to be restricted to populations causing characteristic elongate galls and abnormally elongated floral structures in grasses of the genus *Agrostis*. Our data support Southey's suggestion and the concept of narrow specialisation of seed-gall nematodes, but further show that *A. agrostis* occurs only in one host, *Agrostis capillaries*, and that other *Agrostis* spp. are hosts for another two undescribed *Anguina* species.

3.3. Evolution of gall types

Paramonov (1970) suggested that the ancestral anguinid branch (*S. radicicola*) primarily infected the root system and did not develop further. Subsequently, the primitive anguinid forms moved into the vegetative organs of plants and, finally, occupied the generative organs (Solovyeva and Krall, 1983). The molecular phylogeny gives a relatively clear picture of the evolutionary trends in gall formation in anguinids (Fig. 2): (i) abnormal growth of the infested tissues evolved to true galls, (ii) decreasing of gall size, and (iii) gall formation proceeded from vegetative to generative plant organs. Seed galls evolved at least twice during anguinid evolution, the independent origin of seed gall type is significantly supported from ML tests of our data set (second table of Appendix A). Although changes of gall formation on different organs and gall type could be nonadaptive, the trend in gall evolution appears to suggest an adaptive strategy. There are several mechanisms that could select for infestation of generative organs of plants: (i) a decreasing influence of the parasite on the plant photosynthetic process, (ii) a more efficient and longer association of the parasite to the nutrient flow in the plant, (iii) the use of the same mechanism for dispersion as that of the host plant. Above-mentioned trends in gall evolution, however, were not evidently

marked for other gall-forming organisms, and perhaps, may be specific for nematodes only.

3.4. Cospeciation of anguinids with their host plants

After studying the parasitic relationships of the gall-forming nematodes with their hosts, Krall (1975) concluded that nematode evolution was generally in accordance with that of their hosts. The present molecular data suggest that anguinid groups are generally associated with host plants from the same or related systematic groups belonging to the Poaceae and the Asteraceae. To examine congruence between plant and nematode phylogenies, cospeciation and host switching events, the phylogeny of anguinids parasitising Poaceae and the phylogeny of grasses were compared in a separate analysis. The MP analyses of the nematode sequence alignment with a reduced number of species and the plant sequence alignment resulted a single most parsimonious trees for each of the data sets (Fig. 3, Appendix A). The partition homogeneity test indicated substantial incongruence between the nematode and grass data sets, and revealed that anguinid nematodes and grasses do not have the same history ($P = 0.001$). Thus, strict cospeciation can be rejected for these grass-nematode associations. However, when the nematode
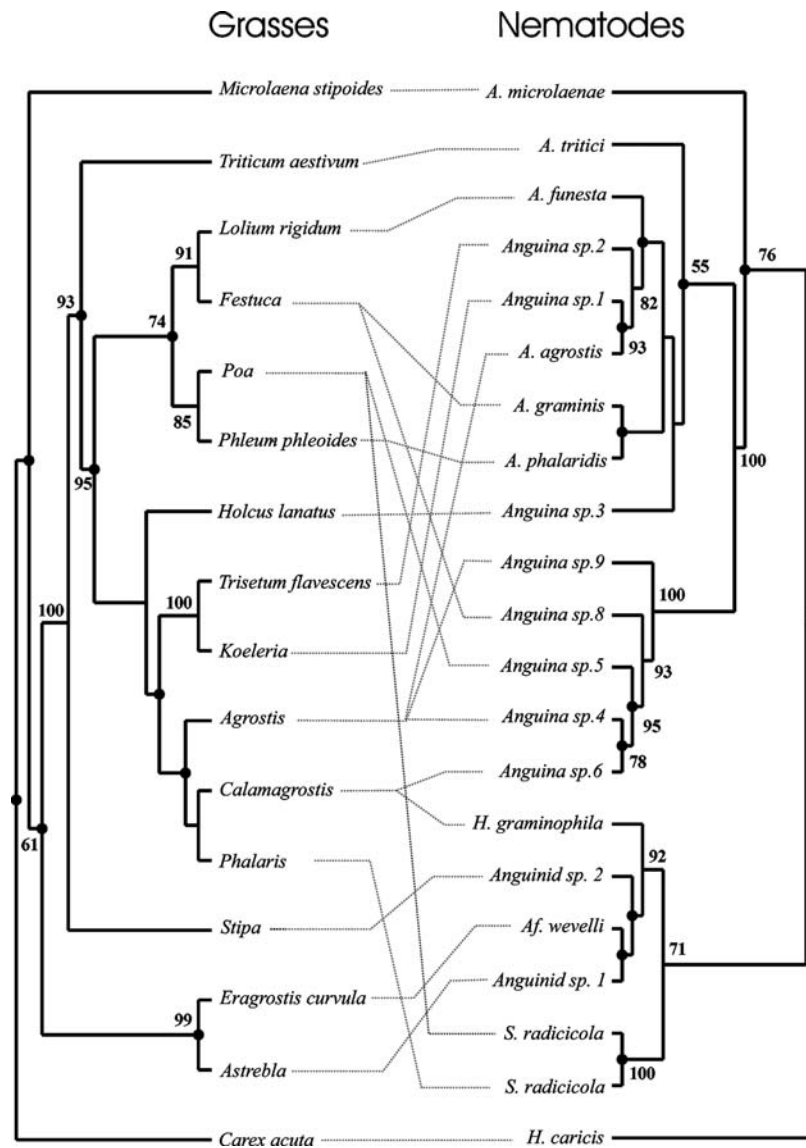

Fig. 3. Comparison of MP trees for anguinids (tree length = 627) and grasses (tree length = 814) based on the ITS sequences. Number above the branches indicates bootstrap percentage; only nodes supported by 50% or greater are shown. ●, nodes matching in the nematode and host trees. Distribution of nematodes on hosts is shown by puncted lines. Plant sequences have the following GenBank Accession Nos. *Astrebla*—AF019838, *Calamagrostis*—AJ306448, *Carex acuta*—AF284992, *Festuca rubra*—AF147141, *Holcus lanatus*—Z96914, Z96915, *Koeleria*—Z96904, Z96905, *Lolium rigidum*—AF171161, *Microlaena stipoides*—AF019791, *Phalaris*—L36522, *Poa*—AF171182, *Stipa*—AF019803, *Trisetum flavescens*—Z96898, Z96899, and *Triticum aestivum*—AJ301799.

tree was mapped onto the grass topology, TreeMap found 126 optimal reconstructions with 12 cospeciations, 4–6 duplications, 1–4 host switches. The level of cospeciation was estimated as 60%. The probability of obtaining the observed number of cospeciation events can not be explained as a result of random establishments of host–parasite associations ($P < 0.001$). The calculated level of cospeciation obtained in the present study is comparable, for example, with that for pollinating wasps and figs (50–64%), and pocket gophers and lice (62.5%) (Lopez-Vaamonde et al., 2001).

The absence of perfect congruence between phylogenies can result from differences in data quality as demonstrated by Clark et al. (2000) and Lopez-Vaamonde et al. (2001). A robust plant phylogeny is not available, thus additional information from other nematode and plant genes and from total evidence analysis is required to test the hypothesis of co-evolution of anguinids with their hosts. On other hand, there are many different ecological and biogeographical scenarios that can result in partial congruence in phylogenetic patterns for partners in symbiotic association, but evidence for such processes for anguinids and their host plants is not available.

The usefulness of ITS sequences for phylogenetic analysis and the evaluation of existing classification in Anguinidae has been demonstrated in this paper. Also, the first molecular evidence is provided for cospeciation between plant nematodes and their host plant. The combined analysis of the ITS region with other genes should enhance our understanding of gall-forming nematode phylogeny and their coevolution with hosts.

Acknowledgments

Appendix A

Nematode species from the subfamily Anguininae used in this study

Species and populations	GenBank Accession Nos.	Plant-host	Gall location	Locality	Source
Anguina Scopoli (1777)					
A. agropyri Kirjanova (1955)	AF396355	*Elymus repens*	Basal stem gall	Tartu, Estonia	E. Krall
A. agrostis (Steinbuch, 1799)	AF396344	*Agrostis capillaris*	Seed gall	Moscow region, Russia	V. Chizhov
A. agrostis	AF396342	*A. capillaris*	Seed gall	New Zealand	G. Yeates
A. agrostis	AF396338	*A. capillaris*	Seed gall	Kasterlee, Belgium	S. Subbotin
A. agrostis	AF396339	*A. capillaris*	Seed gall	Oregon, USA	H. Jensen
A. agrostis	AF396340	*Agrostis* sp.	Seed gall	Kunashir, Sakhalin region, Russia	S. Subbotin
A. agrostis	AF396341	*Agrostis* sp.	Seed gall	Sakhalin, Russia	S. Subbotin
A. agrostis	AF396343	*Agrostis* sp.	Seed gall	Iturup, Sakhalin region, Russia	S. Subbotin
A. australis Steiner (1940)	AF396334	*Ehrharta longiflora*	Leaf gall	Esperance, Western Australia, Australia	I. Riley
A. australis	AF396335	*Eh. longiflora*	Leaf gall	Perth, Western Australia, Australia	G. Yan
A. funesta (Price, Fisher and Kerr, 1979)	AF396347	*Lolium rigidum*	Seed gall	Lochiel, South Australia, Australia	I. Riley
A.funesta	AF396348	*L. rigidum*	Seed gall	Warooka, South Australia, Australia	I. Riley
A.funesta	AF396349	*L. rigidum*	Seed gall	Damboring, Western Australia, Australia	I. Riley
A. graminis (Hardy, 1850)	AF396351	*Festuca rubra*	Leaf gall	Moscow region, Russia	V. Chizhov
A. tritici (Steinbuch, 1799)	AF396353	*Triticum* sp.	Seed gall	Unknown	M. Moens
A. tritici	AF396354	*T. aestivum*	Seed gall	Carnamah, Western Australia, Australia	I. Riley
A. microlaenae (Fawcett, 1938)	AF396333	*Microlaena stipoides*	Leaf gall	Toowoomba, Queensland, Australia	K. Owen
A. phalaridis (Steinbuch, 1799)	AF396352	*Phleum phleoides*	Seed gall	Kaarma, Estonia	E. Krall
A. askenasyi (Bütschli, 1873)	AF396336	*Calliergon* sp.	Terminal stem gall	Tartu district, Estonia	E. Krall

Appendix A (*continued*)

Species and populations	GenBank Accession Nos.	Plant-host	Gall location	Locality	Source
A. askenasyi	AF396337	*Calliergonella cuspidata*	Terminal stem gall	Valga, Estonia	E. Krall
Anguina sp.1	AF396345	*Koeleria gracilis*	Seed gall	Kharkov region, Ukraine	M.G. Kalinichenko
Anguina sp.2	AF396346	*Trisetum flavescens*	Seed gall	Czech Republic	E. Krall
Anguina sp.3	AF396350	*Holcus lanatus*	Seed gall	Wymah, New South Wales, Australia	I. Riley
Anguina sp.4	AF396356	*Arctagrostis latifolia*	Seed gall	Olyutorsk district, Kamchatka, Russia	N. Probatova
Anguina sp.4	AF396357	*Agrostis flaccida*	Seed gall	Shumshu, Sakhalin region, Russia	I.P. Mishin
Anguina sp.5	AF396360	*Poa malacantha*	Seed gall	Mednyi, Commander Islands, Russia	N. Probatova
Anguina sp.5	AF396361	*Poa sibirica*	Seed gall	r. Khatanga, Taimyr, Russia	N. Vargina
Anguina sp.6	AF396358	*Calamagrostis purpurea*	Seed gall	Kamchatka, Russia	N. Probatova
Anguina sp.7	AF396359	*Hierochloe alpina*	Seed gall	Olyutorsk district, Kamchatka, Russia	N. Probatova
Anguina sp.8	AF396362	*Festuca nigrescens*	Seed gall	New Zealand	G. Yeates
Anguina sp.9	AF396363	*Polypogon monspeliensis*	Seed gall	Greenways, South Australia, Australia	T. Bertozzi
Anguina sp.9	AF396364	*Agrostis avenacea*	Seed gall	Collarenebri, New South Wales, Australia	T. Bertozzi
Anguinid sp.1	AF396368	*Astrebla pectinata*	Stem gall	Withdorah, Queensland, Australia	T. Jessop
Anguinid sp.2	AF396316	*Stipa scabra* ssp. *scabra*	Seed gall	Wyperfeld National Park, Victoria, Australia	A. McKay and I. Riley
Afrina Brzeski (1981)					
Af. wevelli (Van Den Berg, 1985)	AF396317	*Eragrostis curvula*	Seed gall	South Africa	D. Peng
Ditylenchus Filipjev (1936)					
D. dipsaci (Kühn, 1857) "A"	AF396321	*Fragaria* sp.	Stem swollen	Moscow region, Russia	V. Chizhov
D. dipsaci "A"	AF396320	*Medicago sativa*	Stem swollen	Island Hiinmag Kassari, Estonia	H. Krall
D. dipsaci "A"	AF396319	*Trifolium pratense*	Stem swollen	Viljandi, Estonia	E. Krall
D. dipsaci "B"	AF396323	*Vicia faba*	Stem swollen	Gharb region, Morocco	S. Amiri
D. dipsaci "C"	AF396322	*Cirsium setosum*	Stem swollen	Moscow region, Russia	V. Chizhov
Heteroanguina Chizhov (1980)					
H. caricis (Solovyeva and Krall, 1982)	AF396311	*Carex acuta*	Large leaf gall	Haapsalu district, Matsalu, Estonia	E. Krall
H. ferulae (Ivanova, 1977) "A"	AF396324	*Ferula Jaeschkeana*	Large leaf gall	Varsob, Tadzhikistan	T. Ivanova
H. ferulae "B"	AF396325	*Ferula* sp.	Large leaf gall	Uzbekistan	Sh. Khuramov
H. graminophila (Goodey, 1933)	AF396315	*Calamagrostis neglecta*	Large leaf gall	Moscow region, Russia	V. Chizhov
H. graminophila	AF396318	*Calamagrostis* sp.	Large leaf gall	Kunashir, Sakhalin region, Russia	S. Subbotin
Mesoanguina Chizhov and Subbotin (1985)					
M. picridis (Kirjanova, 1944)	AF396329	*Acroptilon repens*	Leaf gall	Tadzhikistan	L. Dzhuraeva
M. picridis	AF396326	*Ac. repens*	Leaf gall	Uzbekistan	A. Ryss
M. picridis [(syn. *M. pharangii* (Chizhov, 1984)]	AF396330	*Centaurea leucophylla*	Leaf gall	Kabardino-Balkaria, Russia	V. Chizhov
M. picridis [(syn. *M. kopetdaghica* (Kirjanova and Shagalina, 1969)]	AF396328	*Cousinia onopordioides*	Leaf gall	Gautan, Ashkhabad region, Turkmenistan	L. Shagalina

Appendix A (*continued*)

Species and populations	GenBank Accession Nos.	Plant-host	Gall location	Locality	Source
M. picridis [(syn. *M. varsobica* (Kirjanova and Ivanova, 1968)]	AF396327	*Rhaponticum integrifolia*	Leaf gall	Kondara, Tadzhikistan	L. Dzhuraeva
M. mobilis (Chit and Fisher, 1975)	AF396331	*Arctotheca calendula*	Leaf gall	Urrbrae, Adelaide, Australia	I. Riley and W. Wouts
M. moxae (Yokoo and Choim 1968)	AF396314	*Artemisia rubripes*	Leaf gall	Primorskii territory, Russia	A.S. Eros-henko
M. millefolii (Löw, 1874)	AF396312	*Achillea millefolium*	Leaf gall	Moscow region, Russia	V. Chizhov
M. millefolii	AF396313	*Achillea biebersteinii*	Leaf gall	Kopet-Dag, Bakhardin district, Turkmenistan	L. Shagalina
Mesoanguina sp.	AF396332	*Anaphalis margaritacea*	Stem gall	Sakhalin, Russia	H. Krall
Subanguina Paramonov (1967)					
S. radicicola (Greef, 1872)	AF396366	*Poa annua*	Root gall	Moscow region, Russia	V. Chizhov
S. radicicola	AF396367	*Phalaris arundinaceae*	Root gall	Voru, Estonia	H. Krall
S. radicicola	AF396365	*Poa* sp.	Root gall	Luxemburg province, Belgium	S. Subbotin

Results of Kishino–Hasegawa (KH) and Shimodaira–Hasegawa (SH) tests of alternative hypotheses for Anguinidae phylogeny

Topology evaluated	Best log likelihood (tree number)	KH		SH	
		$\Delta \ln L$	P	$\Delta \ln L$	P
ML tree	−6269.51 (1)	–	–	–	–
MP trees (gaps as missing character)	−6278.59 (14)	−9.08	0.3083	−9.08	0.169
MP trees (gaps as fifth characters)	−6275.48 (4)	−5.97	0.5533	−5.97	0.267
Ditylenchus dipsaci + *A. askenasyi*	−6313.79 (56)	44.27	0.0053*	44.06	0.003*
Monophyly of *Heteroanguina*	−6368.11 (252)	98.60	<0.0001*	98.76	<0.0001*
Heteroanguina graminophila + *H. caricis*	−6299.79 (84)	30.27	0.0072*	30.48	0.010*
Monophyly of *Mesoanguina*	−6293.17 (14)	23.66	0.0291*	23.84	0.021*
Monophyly of Anguininae infesting grasses and sedges (inc. *A. askenasyi*)	−6281.27 (14)	11.75	0.2202	11.67	0.116
Monophyly of seed-gall forming nematodes	−6351.10 (70)	81.60	<0.0001*	83.00	<0.0001*

* User defined tree significantly worse that the best tree at $P < 0.05$.

References

Brzeski, M.W., 1981. The genera of Anguinidae (Nematoda, Tylenchida). Rev. Nematol. 4, 23–34.

Chizhov, V.N., Subbotin, S.A., 1990. Plant-parasitic nematodes of the subfamily Anguininae (Nematoda, Tylenchida). Morphology, trophic specialization, system. Zool. Zh. 69 (1), 15–26 (in Russian).

Clark, M.A., Moran, N.A., Baumann, P., Wernegreen, J.J., 2000. Cospeciation between bacterial endosymbionts (*Buchnera*) and a recent radiation of aphids (*Uroleucon*) and pitfalls of testing for phylogenetic congruence. Evolution 54, 517–525.

Kirjanova, E.S., Krall, E.L., 1971. In: Plant Parasitic Nematodes and their Control, vol. 2. Nauka, Leningrad, p. 522 (in Russian).

Krall, E.L., 1975. Characterisation of host–parasite interrelations of plant-parasitic nematodes of Anguininae with grasses and possibility of study of gall pathogens. In: Problemy Onkologii i Teratoogii Rastenii. Nauka, Leningrad, pp. 72–75 (in Russian).

Krall, E.L., 1991. Wheat and grass nematodes: *Anguina, Subanguina*, and related genera. In: Nickle, W.R. (Ed.), Manual of Agricultural Nematology. Marcel Dekker, Inc., New York, pp. 721–760.

Lopez-Vaamonde, C., Rasplus, J.Y., Weiblen, G.D., Cook, J.M., 2001. Molecular phylogenies of fig wasps: partial cocladogenesis of pollinators and parasites. Mol. Phylogenet. Evol. 21, 55–71.

Page, R.D.M., 1994. Parallel phylogenies: reconstructing the history of host-parasite assemblages. Cladistics 10, 155–173.

Paramonov, A.A., 1970. Fundamentals of plant helminthology. In: Taxonomy of Nematodes of the Superfamily Tylenchoidea, vol. 3. Nauka, Moscow, p. 233 (in Russian).

Posada, D., Crandall, K., 1998. MODELTEST: testing the model of DNA substitution. Bioinformatics 14, 817–818.

Powers, T.O., Szalanski, A.L., Mullin, P.G., Harris, T.S., Bertozzi, T., Griesbach, J.A., 2001. Identification of seed gall nematodes of agronomic and regulator concern with PCR-RFLP of ITS1. J. Nematol. 33, 191–194.

Riley, I.T., Reardon, T.B., McKay, A.C., 1988. Electrophoretic resolution of species boundaries in seed-gall nematodes, *Anguina* spp. (Nematoda: Anguinidae), from some graminaceous hosts in Australia and New Zealand. Nematologica 34, 401–411.

Roy, B.A., 2001. Patterns of associations between crucifers and their flower-mimic pathogens: host jumps are more common than coevolution or cospeciation. Evolution 55, 41–53.

Siddiqi, M.R., 2000. Tylenchida, Parasites of Plants and Insects, second ed. CABI Publishing, Wallingford. 848 pp.

Solovyeva, G.I., Krall, E.L., 1983. The Sedge Leaf Gall Nematode. Nauka, Leningrad. 79 pp (in Russian).

Southey, J.F., 1973. *Anguina agrostis*. C.I.H. Description of Plant-parasitic Nematodes. Set 2, vol. 20, 3 pp.

Sturhan, D., Brzeski, M.W., 1991. Stem and bulb nematodes, *Ditylenchus* spp. In: Nickle, W.R. (Ed.), Manual of Agricultural Nematology. Marcel Dekker, Inc., New York, pp. 423–464.

Swofford, D.L., 2002. PAUP*. Phylogenetic Analysis using Parsimony and Other Methods. Ver. 4. Sinauer Associates, Sunderland, MA. 128 pp.

White, T.J., Bruns, T., Lee, S., Taylor, J., 1990. Amplification and direct sequencing of fungal ribosomal RNA gene for phylogenetics. In: Innis, M., Gelfand, D., Sninsky, J., White, T. (Eds.), PCR Protocols: A Guide to Methods and Amplifications. Academic Press, San Diego, pp. 315–322.

Zheng, J., Subbotin, S.A., Waeyenberge, L., Moens, M., 2000. Molecular characterisation of Chinese *Heterodera glycines* and *H. avenae* populations based on RFLPs and sequences of rDNA-ITS regions. Russ. J. Nematol. 8, 109–113.