

Molecular characterisation of gall-forming nematodes, *Mesoanguina amsinckiae* and *Anguina danthoniae* (Anguinidae: Tylenchida) from California, USA

Ignacio Cid Del Prado Vera¹, Vladimir N. Chizhov² and Sergei A. Subbotin^{2,3}

¹Colegio de Postgraduados, 56230, Montecillo, Mexico

²Centre of Parasitology, A.N. Severtsov Institute of Ecology and Evolution, Russian Academy of Sciences, Leninskii Prospect 33, 117071, Moscow, Russia

³Plant Pest Diagnostics Centre, California Department of Food and Agriculture, 3294 Meadowview Road, 95832-1448, Sacramento, CA, USA
e-mail: sergei.a.subbotin@gmail.com

Accepted for publication 17 October 2018

Summary. Two gall-forming nematodes, *Mesoanguina amsinckiae* and *Anguina danthoniae*, collected in California were molecularly characterised. Phylogenetic relationships of these species with other plant parasitic Anguinidae based on the analysis of the ITS rRNA gene sequences are given as inferred from Bayesian analysis. *Mesoanguina amsinckiae* parasitising *Amsinckia* spp. from the family Boraginaceae formed the lineage within the clade with *Ditylenchus* and *Heteroanguina*. The position of *A. danthoniae* is not well resolved with the genus *Anguina*. The molecular dataset does not contradict the proposal of synonymisation of the genus *Cynipanguina* with *Anguina*.

Key words: *Amsinckia*, *Anguina*, *Danthonia californica*, ITS-rRNA, *Mesoanguina*, phylogeny.

The family Anguinidae Nicoll, 1935 includes mycophagous and plant-parasitic nematodes. The latter are obligate specialised parasites of higher plants, mosses and seaweeds on which they often induce swellings and galls (Subbotin & Riley, 2012). Anguinids are found in all continents except for Antarctica. Several species have been found and described from California, USA including *Mesoanguina amsinckiae* (Steiner & Scott, 1934) Chizhov & Subbotin, 1985 and *Anguina danthoniae* (Maggenti, Hart & Paxman 1973) Brzeski, 1981.

Mesoanguina amsinckiae was discovered on the host plant *Amsinckia intermedia* and firstly described by Steiner and Scott (1934) under the name *Anguillulina dipsaci* var. *amsinckiae*. Godfrey (1940) referred to the nematode as *Ditylenchus dipsaci* var. *amsinckiae* and Filipjev and Schuurmans Stekhoven (1941) named it as *Ditylenchus amsinckiae*. Thorne (1961) reclassified the nematode as a species of *Anguina*. Brzeski (1991) and Siddiqi (1996, 2000) also listed this species in this genus. Chizhov and Subbotin (1985) transferred it to the genus *Mesoanguina*.

Anguina danthoniae was firstly described under the name *Cynipanguina danthoniae* Maggenti, Hart & Paxman 1973 from California oatgrass, *Danthonia californica*, from central California coast. The nematode induces spherical galls ranging in size from 0.5 to 3.0 mm and varying in colour from green to straw yellow (Maggenti *et al.*, 1973). Maggenti *et al.* (1973) proposed a new genus for this species considering that this species differed from *Anguina* and all other anguinid-like nematodes by having a digit-like process extending from the posterior glandular portion of the pharynx and having spicules with the discrete suture between calamus and lamina. The genus *Cynipanguina* was synonymised with *Anguina* by Brzeski (1981). Later this synonymisation was supported by Chizhov and Subbotin (1985, 1990), Krall (1991) and Siddiqi (1996, 2000); however, it was rejected by Fortuner and Maggenti (1987).

The goal of this work is to provide molecular characterisations of *A. danthoniae* and *M. amsinckiae* collected in California, USA.

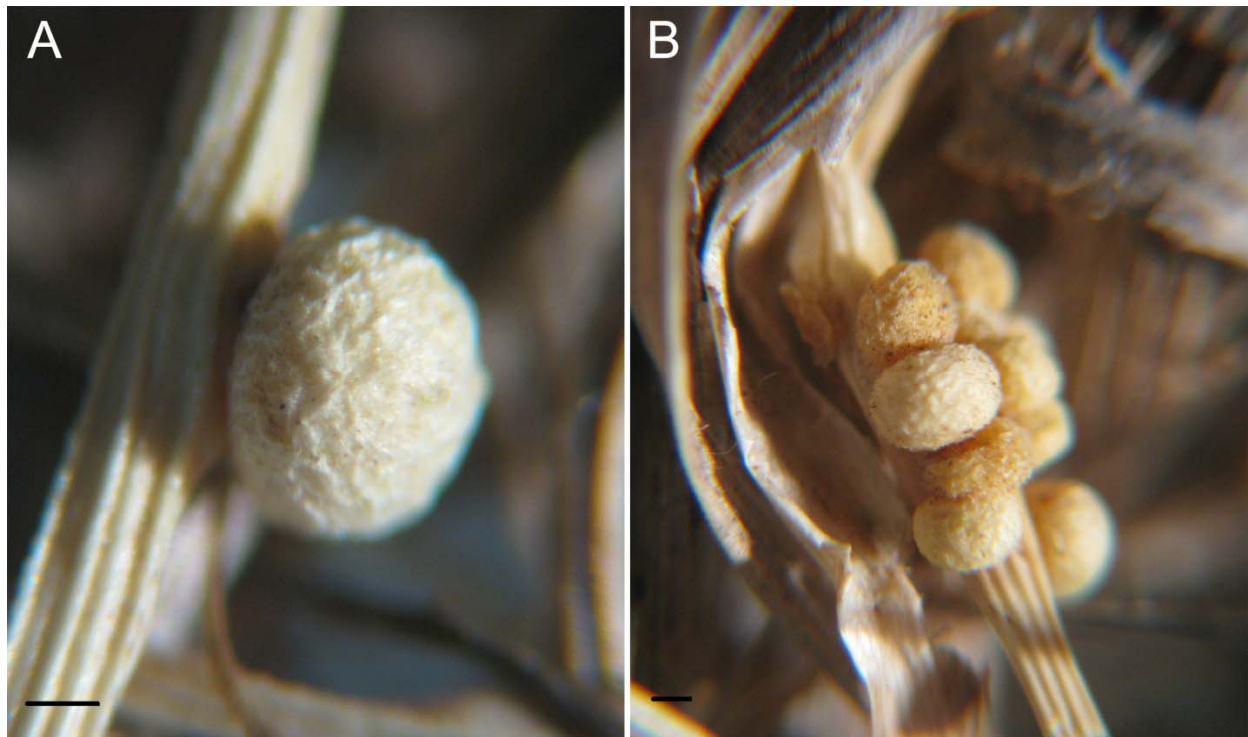


Fig. 1. Leaf galls of *Anguina danthoniae* (Scale = 0.5 mm).

MATERIAL AND METHODS

Nematode populations, DNA extraction, PCR, and sequencing. Dried galls of *Danthonia californica* with *Anguina danthoniae* (Fig. 1) collected by the first author from the type locality and dried *Amsinckia* plant fragments infected by *Mesoanguina amsinckiae* obtained from the CDFA collection were used in this study. DNA was extracted from several nematodes using proteinase K protocol (Tanha Maafi *et al.*, 2003). The ITS rRNA gene was amplified with the forward TW81 (5'-GTT TCC GTA GGT GAA CCT GC-3') and the reverse AB28 (5'-ATA TGC TTA AGT TCA GCG GGT-3') primers as described by Tanha Maafi *et al.* (2003). PCR products were purified using QIAquick PCR Purification Kit (Qiagen) and directly sequenced. New sequences were deposited in the GenBank under accession numbers: MK032869 and MK032870.

Phylogenetic analysis. Sequences of ITS rRNA genes of *M. amsinckiae* and *A. danthoniae* were aligned using Clustal_X 1.83 (Thompson *et al.*, 1997) with published gene sequences of anguinid nematodes (*e.g.*, Powers *et al.*, 2001; Subbotin *et al.*, 2004, 2005; Bertozzi & Davies, 2009; Mobasserri *et al.*, 2017; Barrantes-Infante *et al.*, 2018). Alignment was analysed with Bayesian

inference (BI) using MrBayes 3.1.2 (Ronquist & Huelsenbeck, 2003). Models of DNA evolution for BI were estimated using MrModeltest 2.2 and the best-fit was chosen using the Akaike Information Criterion (Nylander, 2002). BI analysis under the GTR + G model was initiated with a random starting tree and run with the four Metropolis-coupled Markov chain Monte Carlo (MCMC) for 3×10^6 generations. The MCMC were sampled at intervals of 100 generations. After discarding burn-in samples and evaluating convergence the remaining samples were retained for further analysis. The topologies were used to generate a 50% majority rule consensus tree. Posterior probabilities (PP) more than 70% are given for appropriate clades.

RESULTS AND DISCUSSION

The ITS rRNA gene sequence alignment contained 51 sequences including sequences of *Orrina phyllobia* and *Cephalenchus cephalodiscus* selected as outgroup species. It was 829 bp in a length. *Mesoanguina amsinckiae* clustered with a moderate statistical support (PP = 81%) in the clade containing *Ditylenchus* species and *Heteroanguina ferulae*. *Anguina danthoniae* formed a separate lineage and its relationships with other anguinids were unresolved (Fig. 2).

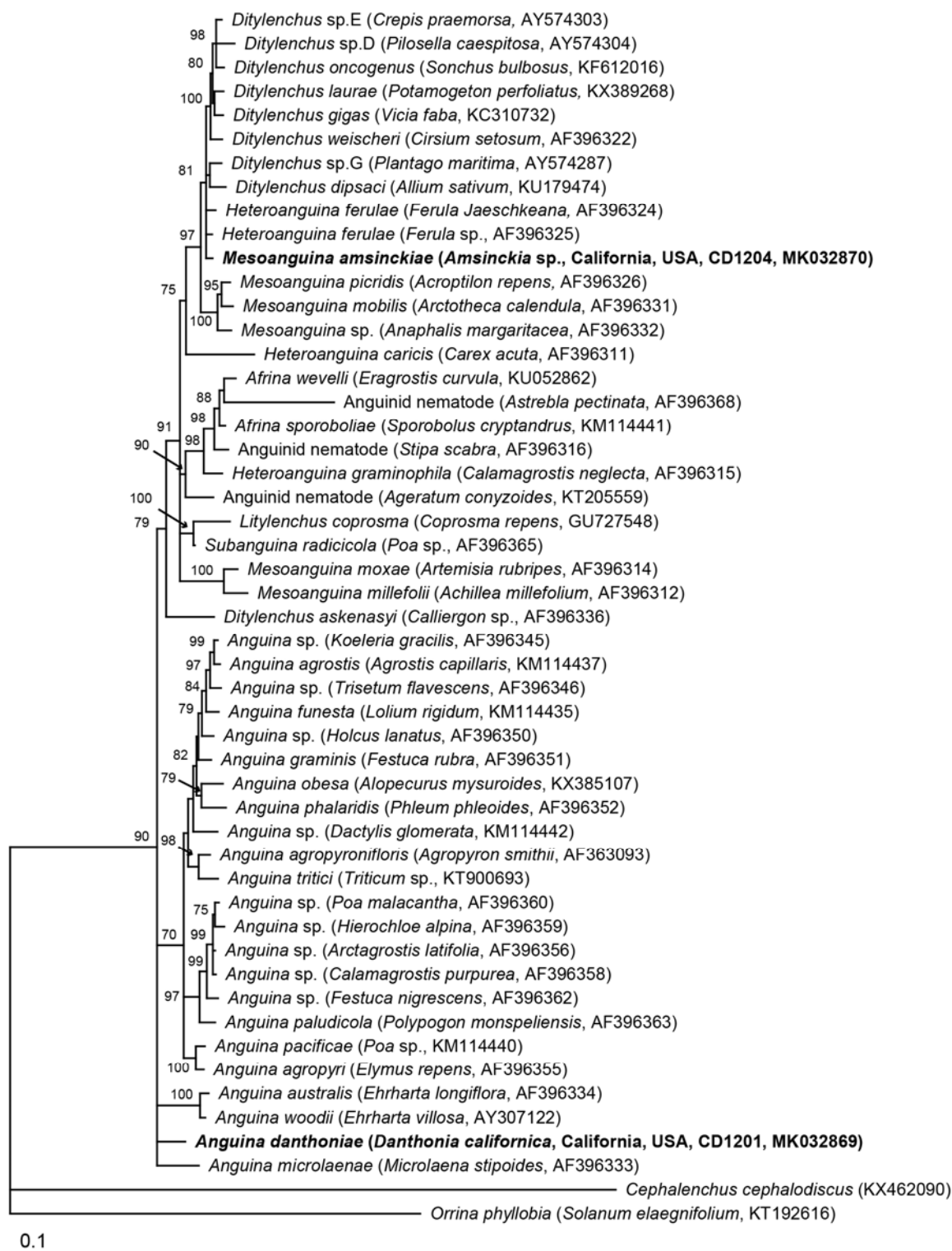


Fig. 2. Phylogenetic relationships within some species of Anguinidae as inferred from Bayesian analysis using the ITS rRNA gene sequence data set under the GTR + G model. Posterior probabilities of over 70% are given for appropriate clades. Newly obtained sequences are indicated in bold.

Topology of the obtained tree is congruent with those published by Subbotin *et al.* (2004) and Barrantes-Infante *et al.* (2018). Subbotin *et al.* (2004) noticed a paraphyly of the genus *Mesoanguina* parasitising Asteraceae and found that the studied species of this genus clustered into two distinct groups in an agreement with their peculiarities of morphology and host plant range. In our tree the genus *Mesoanguina* parasitising Asteraceae is also divided in these two groups; moreover, *M amsinckiae* parasitising *Amsinckia* spp. from the family Boraginaceae formed the lineage within the clade with *Ditylenchus* and *Heteroanguina*. Thus, the revision of the non-monophyletic genus *Mesoanguina* is still needed to transfer these groups in different taxon units.

The genus *Anguina* is monophyletic according to Subbotin *et al.* (2004) and Barrantes-Infante *et al.* (2018). Two species, *A. australis* and *A. microlaenae* inducing leaf and stem galls in plants of the subfamily Bambusoideae, occupied a basal position within *Anguina* species parasitising Poaceae in the tree published by Subbotin *et al.* (2004) and Barrantes-Infante *et al.* (2018). In the present tree the positions of *A. australis* plus *A. woodi* and *A. microlaenae* as well as *A. danthoniae* are not resolved with the genus *Anguina*. However, the monophyly of the genus *Anguina* including *A. danthoniae* is not rejected based on this ITS rRNA topology. Thus, the molecular dataset does not contradict the proposal to synonymise the genus *Cynipanguina* with *Anguina* made by Brzeski (1981).

Comprehensive morphological and molecular analyses are still needed to understand relationships within anguinid nematodes and replace the present artificial classification by a natural one reflecting evolutionary trends in this group (Subbotin & Riley, 2012).

ACKNOWLEDGEMENTS

This work was supported by a FarmBill19 grant, CDFA Agmt no. 18-0346-000-FR.

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Cid Del Prado Vera, I., V.N. Chizhov and S.A. Subbotin. Молекулярная характеристика галлообразующих нематод, *Mesoanguina amsinckiae* и *Anguina danthoniae* (Anguinidae: Tylenchida) из Калифорнии, США.

Резюме. Приводятся данные по нуклеотидным последовательностям двух видов галлообразующих нематод *Mesoanguina amsinckiae* и *Anguina danthoniae*, обнаруженных в Калифорнии, США. По результатам Байесова анализа последовательностей ITS рДНК определены филогенетические взаимоотношения этих видов с другими паразитирующими на растениях видов семейства Anguinidae. Вид *Mesoanguina amsinckiae*, паразитирующий на *Amsinckia* spp. из семейства Boraginaceae, показал родство с родами *Ditylenchus* и *Heteroanguina*. Взаимоотношения вида *A. danthoniae* с другими видами рода *Anguina* остаются неразрешенным. Молекулярные данные не противоречат предложенной синонимизации рода *Cynipanguina* с *Anguina*.
