A new gall midge (Diptera, Cecidomyiidae) as a potential candidate for biological control of the invasive plant *Cortaderia selloana* (Poaceae)

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Abstract

A gall midge (Diptera, Cecidomyiidae) is reported here for the first time from spikelets of *Cortaderia selloana*, a prominent invasive grass in southern Europe. The insect is described as a new genus and species, *Spanolepis selloanae* Gagné. Based on morphological and molecular analyses, the new genus and species are tentatively placed within the supertribe Lasiopteridi and tribe Dasineurini. Its effects on seed production were studied in order to ascertain its effectiveness in limiting sexual reproduction of the invasive plant species. The larvae of *S. selloanae* feed on the ovaries with a mean seed depletion of 74% in the population studied in northwest Spain. The new species is a potential candidate for a biological control agent against *C. selloana*.

Key words: Lasiopteridi, Dasineurini, biological control, invasive alien species, DNA barcoding

Introduction

The Enemy Release Hypothesis (ERH) states that the lack of natural enemies provides invasive alien species with a competitive advantage, partially explaining the success of invasive species in the new environments (Keane and Crawley 2002). Classical Biological Control consists on the intentional release of natural enemies of non-native species, either predators, herbivores, parasites or pathogens, to counteract their expansion in invaded ecosystems (Van Driesche et al. 2010, Schwarzländer et al. 2010). The use of biological control agents in invasive species management has proved to be an effective method when they affect sensitive stages of the life cycle. However, the release of natural enemies obtained in the native areas of an invader has raised concerns among researchers because they may become harmful to native species or disrupt ecosystem networks (Messing 2000, Simberloff and Stiling 1996). Despite these concerns, biological control is now widely recognized as the most reliable tool for long-term control of invasive species (Sheppard et al. 2006).

In invasive plants, insects are some of the most effective biological control agents (Blossey 1995). Insects that reduce seed production are especially effective in plants in which sexual reproduction is the main form of plant regeneration (Paynter et al. 1996, Sheppard et al. 2006). Gall midges (Diptera, Cecidomyiidae) are some of the most important plant-feeding species worldwide (Gagné 1989, 2017). Most species appear to be host-specific, what makes them potentially suitable for biological control with a low risk to the native flora. *Dasineura dielsi* Rübsaamen and *Dasineura rubiformis* Kolesik are two examples of Australian seed-feeding species currently used as biological control agents on two invasive species of *Acacia* (Mimosiidae) in South Africa (Impson et al. 2013, Post et al. 2010). Both species show a life cycle synchronized with the phenology of the host plant, whereby females lay their eggs in the flowers before or immediately

following anthesis. The high host specificity of these species has been demonstrated, including tests in other *Acacia* species (Adair 2005).

Cortaderia selloana (Schult. & Schult.f.) Asch. & Graebn. (Poaceae) is an aggressive invader in the Mediterranean and Atlantic oceanic areas of western Europe (Tarabon et al. 2018). It is a perennial grass with large vegetative tussocks of long leaves serrated at their margins. In late summer, adult plants of over 2-3 years develop tall stalks with terminal flowering heads that may reach 4 metres in height (Harradine 1991, Herrera and Campos 2006). The species is described as gynodioecious, with hermaphrodites bearing well developed anthers, and female plants with viable ovaries (Knowles and Ecroyd 1985). Hermaphrodite plants act mainly as pollen donors, and produce only limited numbers of viable seeds (Connor 1973). The dispersal unit is the complete floret. The female florets have long hairs on the glume that aid in long distance dispersal, as they are dispersed by the wind (Herrera and Campos 2006, Saura-Mas and Lloret 2005). A single plant can produce up to 800,000 viable seeds per season (Saura-Mas and Lloret 2005). The species invasive behaviour relies on its extremely effective sexual reproduction, suggesting that control methods should focus on preventing seed production and early establishment of seedlings (Fagúndez and Lema 2019).

Cortaderia selloana is native to Argentina, Uruguay and Brazil (Connor 1973, Lambrinos 2001, Harradine 1991, Herrera and Campos 2006). It was introduced into European gardens in the nineteenth century, and from there to other places like California and New Zealand (Lambrinos 2001, Harradine 1991, Okada et al. 2007). Due to the abundance of seeds produced and ability to colonize disturbed environments, the plant has become one of the most successful invaders in South-West Europe (Nentwig et al. 2018), and the Spanish government has promoted a national strategy to tackle its invasion (MTE 2018). Negative effects of *C. selloana* include the formation of impenetrable monospecific

stands, increasing the risk of wildfires, and the colonization of natural wetland areas threatening native species and natural habitats (Fagúndez and Barrada 2007, Herrera and Campos 2006, Saura-Mas and Lloret 2005).

During a risk assessment analysis of *Cortaderia selloana*, we searched the plant for possible biological control agents. The gall midge newly described in this paper was discovered in a large population of *C. selloana* in the outskirts of the city of A Coruña in Galicia, NW Iberian Peninsula, one of the areas most heavily invaded in Galicia (Pardo-Primoy and Fagúndez 2019). Larvae feed on the developing ovaries and replace the seeds at the time of dispersal. This potential control agent is, to our knowledge, the first to be proposed for *C. selloana* (Herrera and Campos 2006, MTE 2018). The new gall midge is distinctive for characters that separate it from other species known from other grasses, and the genus is unique among the large, heterogeneous tribe Dasineurini (*sensu* Dorchin et al. 2019). Within this clade, recent studies have acknowledged the paraphyly of the large genus *Dasineura* and the need for a comprehensive revision of the tribe (Dorchin et al. 2019, Gagné 2017, 2018, Sevcik et al. 2016, Sikora et al. 2019). Our description includes the "barcode" mitochondrial DNA sequence as a trait of the new taxon. The recognition of the new gall midge as a monotypic genus is preliminary supported by a phylogenetic analysis including all genera within Dasineurini with available homologous sequences on public databases.Lastly, we investigated the effects of the gall midge on the life cycle of the plant and the gall midge, in order to gather essential information on their phenology and reproductive performance.

Materials and methods

Cortaderia selloana and insect sampling

100 The Zapateira area is located in the outskirts of the city of A Coruña, on the Atlantic coast
101 of Galicia, NW Iberian Peninsula (Fig. 1). The area is densely occupied by commercial
102 facilities, the University of A Coruña campus, a hospital, schools and housing. It is
103 heavily invaded by *C. selloana*, which occurs along roadsides, train lines and worklots,
104 and in nearby fields and *Eucalyptus*-forested areas (Pardo-Primoy and Fagúndez 2019).
105 In 2016, larvae and adults of the gall midge were discovered by the first author while
106 searching for potential natural enemies of the invasive plant, and gathering seeds for a
107 germination experiment (Fagúndez and Lema 2019). In October 2017, a population with
108 over one hundred adult plants of *C. selloana* located on the side of a secondary road was
109 surveyed. Twenty-seven plants were randomly selected and labelled for monitoring. We
110 recorded the sex of each plant after observations in the laboratory, and established the sex
111 ratio for the population. In female plants, gall midge larvae were found within each floret,
112 replacing the caryopsis. The prevalence of the larvae was calculated by the ratio of larvae
113 to fruit (L:V) from a subset of random florets examined in each plant. We counted only
114 apparently viable, well developed seeds. Empty florets with undeveloped ovaries were
115 not considered. A mixture of twenty apparently viable seeds were set for germination in
116 a sealed petri dish with soaked filter paper under indoor temperatures and natural light
117 conditions.
118
119 To assess the phenology of the gall midge and the host plant, ten plants of the same
120 population were selected and labelled again in 2018. Samples of each plant were collected
121 at five different times between August and November 2018. In female plants, each floret
122 was classified as i) flower (viable stigmas developed); ii) fruit (caryopses within the
123 florets) and; iii) dispersion (free florets with a fully developed caryopsis). The percentage
124 of each phenophase was estimated, considering all spikelets, therefore the accumulated

125 percentage was below 100% when some of the florets were found empty. In the male
126 plants, florets were classified as pollinating (visible pollen in the developed anthers) and
127 degenerate (brown empty anthers). The presence of *S. selloanae* was classified as adults,
128 pupae or larvae.
129 From samples gathered in October 2017, larvae were collected and stored in paper bags.
130 From 2018 samples, we collected adults, pupae and larvae directly from the plants by
131 removing large portions of the inflorescences after covering with a paper bag. Individuals
132 for identification were preserved in 70% ethanol.
133
134 *Molecular methods*
135 Genomic DNA was extracted from eight larvae and nine adults using a commercial kit
136 (High Pure PCR Template Preparation Kit, Roche) following the manufacturer's
137 instructions. We sequenced up to 699 bp of the 5′ end (barcoding region) of the
138 mitochondrial cytochrome c oxidase subunit I (COI) gene, corresponding to positions
139 [1176-1874] of the mitochondrial genome of *Mayetiola destructor* (Diptera:
140 Cecidomyiidae) (accession number GQ387648.1). Amplifications were carried out in 30
141 µL volumes containing 1X PCR Buffer (5PRIME), 3 mM MgCl2, 1U TaqDNA
142 Polymerase (5PRIME), 0.2 mM of each dNTP, 0.3 µM of each primer (CeciLCOf (5'-
143 TTC TAC TAA TCA TAA AGA TAT TGG-3' modified from LCO-1490 by Folmer et
144 al. 1994) and CeciNancyr (5'-CCW GGT AAA ATT AAA ATA TAA ACT TC-3',
145 modified from C1-N-2191 by Simon et al. 1994)), and approximately 40 ng of DNA.
146 Reaction conditions consisted of 2 min 95 °C followed by 35 cycles of 45 sec 94 °C; 45
147 sec 50 °C; 45 sec 68 °C and, lastly, a final extension of 7 min at 68 °C. Amplified
148 fragments were purified and then bidirectionally sequenced in a 3130xl genetic analyzer

149 (Applied Biosystems). Inspection of electropherograms and alignment of overlapping
150 sequences were performed in CODONCODES 3.7.1.1 (CodonCode, USA).
151 The haplotypes obtained were compared with records available in the NCBI Nucleotide
152 database using BLASTN 2.9.0+ (Zhang et al. 2000) optimised for highly similar
153 sequences (megablast). We accessed the database on 29 April 2020.
154 We investigated the phylogenetic relationships of haplotype 1 of *Spanolepis selloanae*
155 within the tribe Dasineurini using Maximum parsimony (MP), Maximum Likelihood
156 (ML), and Bayesian Inference (BI). We included 38 out of the 42 COI records analysed
157 by Dorchin et al. (2019), the aforementioned records of *Macrolabis fagicola* (JQ684878)
158 and *Janetiella glechomae* (KR740388), a record from *Mayetiola destructor*
159 (GQ387648.1:1145-2680), and haplotype 1 of *Spanolepis selloanae*. The resulting trees
160 were rooted with homologous sequences from two members of the supertribe
161 Lasiopteridi: *Asteromyia carbonifera* (Alycaulini) and *Lasioptera arundinis*
162 (Lasiopterini) (Accession Nos. MN191258 and MN191311, respectively) (see Dorchin et
163 al. 2019). Purging the data produced a final alignment of 44 sequences of 612 bp.
164 We conducted heuristic searches for MP analyses (TBR branch swapping and partial
165 deletion of missing data). The robustness of the topologies was examined after 1000
166 bootstrap replicates. MP calculations were performed using MEGAv10.0.5 (Kumar et al.,
167 2018). The best-fit model of nucleotide substitution was identified using jMODELTEST
168 2 (Darriba et al. 2012) following the Bayesian Information (BIC). Accordingly, we
169 calculated the ML phylogeny using the TPM2uf + I + G model as implemented in the
170 RAxML-NG BlackBox v0.9.0 (Kozlov et al., 2019). Node supports were assessed using
171 the automatic bootstrapping option, which resulted in 200 replicates. High support was
172 interpreted with >70% bootstrap values, whereas low/moderate support corresponded to
173 50-70%.

We conducted BI analyses using MrBayes v3.2.7 (Ronquist et al., 2012). In this case, the substitution model applied was GTR + I + G (the third best model according to jMODELTEST). *Asteromyia carbonifera* was selected as outgroup. We ran two independent analyses, each with four simultaneous Monte Carlo Markov chains (MCMC) for 17 million generations. The chain was sampled every 1000th generation. Convergence was indicated by the average standard deviation of splits (< 0.01). Stationarity was confirmed after summarising the sampled parameters values (discarding 25% of the samples) by a "white noise" plot of loglikelihood scores, PSRF values of 1.000 for all parameters as well as verification of suitable effective sample size (ESS > 400) for all parameters in TRACER v1.7.1 (Rambaut et al., 2018). We obtained the consensus tree with branch length and clade credibility (Bayesian Posterior Probability, BPP) after removing the first 10% samples. High support was interpreted when BPP > 0.95, whereas moderate/low support corresponded to 0.90-0.95 values. Readers are referred to Online Resource 1 for further details on the phylogenetic analyses.

Morphological methods

Adults and immature stages were preserved in 70% ethanol and subsequently mounted by RJG in Canada balsam using the technique outlined in Gagné (1989). Terminology for adult morphology follows Gagné (2018), and that for larval and pupal morphology follows Gagné (1989). Line drawings of Fig. 5 were made by RJG with the use of a camera lucida attached to a Wild phase contrast microscope. Most setae become lost in the mounting process, but the illustrations show their sockets in their actual placement and number, and any setae are drawn to their approximate actual length and thickness.

Results

Phenology of Cortaderia selloana

Data from the two years of this study were combined to provide basic data on plants from the population studied. The 27 plants studied in 2017 were 15 males and 12 females (sex ratio 1.25 for males). The development of flowering heads started in mid-August. Each plant had a mean of 8.6 panicles per plant (rank 2 – 21; N=27). No statistical differences were observed for the number of panicles for male and female flowers (t_{20}=0.176, p=0.86). Flowering started in early September and the peak lasted for c. 15 days (Fig. 2). Fruiting and dispersal lasted for nearly a month. After mid-November, the flowering heads remain in the plant but the number of fertile florets is very low (Fig. 2).

Phenology of Spanolepis selloanae

The mean prevalence of the larvae per plant was 74% (rank 58.6 – 87.5; N = 12), meaning the larvae-fruit ratio (L:F) was approximately 3:1. Germination of the intact seeds was 100%. T50, the time for the germination of 50% of the seeds, was only eight days but some seeds took as long as 69 days to germinate.

Adults of the gall midge were found during a very short period of time. Nearly all were recorded in one visit on September 19th, immediately after the flowering peak of the host plant (Fig. 2). Pupae were also recorded in that visit, but their exuviae persisted longer. Larvae were first detected on September 19th in low numbers, and afterwards followed the same pattern as mature seeds (Fig. 2). All larvae collected were third (last) instars; the two earlier instars were no evident.We also found a wasp of the genus *Aprostocetus* Westwood (Hymenoptera: Eulophidae), most probably a parasitoid of *S. selloanae*.

Molecular analyses

Two new haplotypes of the gall midge were found and submitted to Genbank. Haplotype 1 (699 bp, accession number MT511669) was observed in all larvae and six adults. Haplotype 2 (667 bp, MT511670) was found only in three adults. It is therefore proved that the larvae and adults we studied belong to the same species. Both haplotypes differed in a single non-synonymous substitution at position 357 of the COI gene (reference: accession number GQ387648.1:1145-2680). The BLAST search produced no identical match for any of the two haplotypes. The most similar sequences to both haplotypes were *Macrolabis fagicola* (Barnes) (JQ684878) with regard to total score (915). With regard to percentage of identity, *Rhopalomyia protrahenda* (De Stefani) (MN191340) was the best match to haplotype 1 (90.6), whereas three records of *Janetiella glechomae* (Kieffer) (KR740388, KR743364, KR956680) were the best matches to haplotype 2 (90.69).

The phylogenetic results placed *Spanolepis selloanae* in a single branch within a large polytomy when using the BI (Fig. 3) and MP algorithms (Online resource 1, Fig. 6). The ML method grouped *S. selloanae* with *Dasineura oleae*, but this node received no support (Online resource 1, Fig. 7).

Taxonomy

Spanolepis Gagné, new genus (Figs. 4-5)

Diagnosis. The genus is distinct among all other known Dasineurini by the lack of scales on the adult thoracic sclerites and abdominal terga and sterna, by the very short setae on the female cerci except for the four thickened apical setae (Fig. 5I), and on the larva, the loss of a spatula, one of the two triplets of lateral papillae (Fig. 5L), and four of the eight terminal papillae Fig. 5M).

Description. *Adult*. Female head (Fig. 4A): Eye facets circular, nearly contiguous on ventral two-thirds of eye, 1/2 to whole eye facet apart on dorsal third, eye bridge 3 facets long,

facets at bridge 1 to 2 facets diameter apart. Antenna: male flagellomeres 12 (n=10), all but last with long necks (Fig. 5A); female flagellomeres, 10-11 (n=10), without necks (Fig. 5B). Labella hemispherical, with several stout setae. Palpus 4-segmented with scattered setae and no scales.

Thorax: Wing (Fig. 4B): C broken beyond junction with R_5; R_5 reaching C before wing apex; Rs evanescent; wing fold barely evident; M_4 and CuA forming a fork; scales on membrane sparse. Scutum with 4 discrete longitudinal rows of setae without scales intermixed; median rows mostly single, not reaching scutellum posteriorly, lateral rows sparse with 10-12 setae, mostly placed near midlength of scutum. Scutellum with about 10 dorsal setae. Anepimeron with 6-8 setae, remaining pleural sclerites bare. Acropod (Fig. 5C): claws strongly curved beyond midlength, with basal tooth; empodia as long as claws; pulvilli diminutive.

Male abdomen (Fig. 5D-H): First through sixth tergites rectangular, each with anterior pair of trichoid sensilla, a single, sparse, medially interrupted row of setae along posterior margin, 2-3 lateral setae, and no scales; seventh tergite unsclerotized posteriorly, with only anterior pair of trichoid sensilla, 2-3 lateral setae and no scales; eighth tergite pigmented only anteriorly, with pair of anterior trichoid sensilla the only vestiture. Second through seventh sternites rectangular, with mostly single horizontal row of posterior setae, single to double horizontal row of setae at midlength, pair of closely adjacent trichoid sensilla anteriorly and no scales; eighth sternite with anterior trichoid sensilla missing, midlength and posterior setal rows commingling. Terminalia (Fig. 5E-G): cerci nearly triangular with a few setae apically; hypoproct about as wide as a cercus, divided apically into 2 narrow lobes, each with a pair of setae; aedeagus with convex apex, reaching end of hypoproct; gonocoxite cylindrical laterally, its mediobasal lobe broad, subdivided, the dorsal part of the lobe short, hemispherical, elongate-microtrichose, the ventral part longer, closely

sheathing much of one side of the aedeagus, mostly microtrichose, terminating in apical, short-cylindrical, glabrous appendix tipped with two short setae; gonostylus tapering from base, glabrous and carinate dorsally, microtrichose nearly to apex ventrally, glabrous and carinate beyond.

Female abdomen (Fig. 5I-J): First through seventh tergites rectangular, all with anterior pair of trichoid sensilla, single row of posterior setae interrupted medially except on sixth, with no lateral setae and no scales; seventh tergite square, much narrower than sixth, with sparse row of setae along posterior margin and pair of trichoid sensilla; eighth tergite divided into two narrow, elongate sclerites about twice as long as seventh tergite, each with trichoid sensillum near anterior end and 1-2 setae near posterior end. Second to seventh sternites as for male; eighth sternite not apparent. Ovipositor elongate, protrusible, evenly microtrichose and sparsely short-setose to cerci, protrusible part approximately 5 times length of sixth tergite, with narrow, faintly pigmented dorsolateral sclerite along length; cerci fused, evenly cylindrical to rounded apex, with 4 thick setae with large sockets near apex, elsewhere with scattered short setae no longer than width of their sockets and barely longer than covering microtrichia; hypoproct narrow, nearly twice as long as wide, with 2 distal setae.

Pupal exuviae (Fig. 5K). Head and thorax brown, abdomen hyaline. Antennal bases rounded, not differentially sclerotized or modified into anteriorly pointed projections; with single papilla with seta, situated mediobasally. Vertex on each side with long seta situated on conspicuously raised base. Face smooth, without lobes, with single papilla with setae and 1-2 papillae without setae anterior to clypeus and triplet of papillae on each side near palpal bases, 1 with seta. Prothoracic spiracle elongate. Abdominal terga, pleura and sterna evenly covered with short spicules.

297 *Larva*. Third instar (Figs. 3C, 4L-M). Head capsule hemispherical, cephalic apodemes
298 about half length of head capsule, antennae about twice as long as wide. Integument white
299 except posterior segment darkened, entirely covered with pebbled verrucae except along
300 narrow spiculose band surrounding the 4 ventral papillae on abdominal first through
301 seventh segments. Spatula absent. Papillar pattern basic for Lasiopteridi except only one
302 triplet of papillae present on each side of thoracic segments (Fig. 5L) and 4 terminal
303 papillae (Fig. 5M).
304 Type species, *Spanolepis selloanae* Gagné.
305 Etymology. The name *Spanolepis* combines the Greek words for sparse and scales with
306 reference to the lack of scales on the head and thoracic and abdominal sclerites and their
307 paucity elsewhere on the adult body. The gender is feminine.
308 Remarks. *Spanolepis* belongs to the reconstructed tribe Dasineurini (Dorchin et al. 2019).
309 According to the key for Nearctic species in Gagné (2018), *Spanolepis* resembles
310 *Dasineura* Rondani in the following ways: antennae have a variable number of
311 flagellomeres within a species; male flagellomeres, except for the apicalmost, have a
312 single basal node and distinct apical neck, while those of the female have almost no neck
313 beyond the node; the costal wing vein is broken just posteriad of its juncture with the R_5
314 vein, which terminates anterior to the wing apex; claws are robust, curved beyond
315 midlength, and have a basal tooth; empodia are usually approximately as long as the claws
316 and pulvilli are about 1/3 the length of the claws; the gonocoxite has a tapered mediobasal
317 lobe that is closely juxtaposed to the side of the aedeagus; the female eighth tergite is
318 usually completely divided longitudinally into two separate sclerites; the ovipositor is
319 elongate-protrusible and its cerci are fused to form a single lobe (Gagné 2018). The new
320 genus is distinct from *Dasineura* as well as all other Dasineurini for the following derived
321 attributes: thoracic and abdominal sclerites lack scales, although narrow scales are present

but sparse on legs, wings and abdominal pleura; the male gonocoxal mediobasal lobe terminates in a single, large, glabrous, lobe; the female fused cerci have four short, thickened setae that are especially prominent because the remaining scattered setae are barely longer than the width of their sockets; the larva lacks a spatula and has but one triplet of lateral setae instead of two on each side of the thoracic segments and only four instead of eight papillae on the terminal segment.

***Spanolepis selloanae* Gagné, new species** (Figs.4-5)

Description. *Adult*. Female head as in Fig. 4A. Antenna: scape with 3-4 setae ventrally; pedicel circled with 7-9 setae shorter than those of scape; male flagellomeres 12 (n=10), all but last with long necks (Fig. 5A); female flagellomeres, 10-11 (n=10), without necks. Frons with 10-12 setae. Wing (Fig. 5B): length in males, 1.4-1.5 mm (n=10); in females, 1.5 mm (n=10). Male abdomen as in Figs. 4D-H. Female abdomen as in Figs. 4I-J.

Pupal exuviae as in Fig. 5K.

Larva. Third instar (Fig. 4C): Length 1.3-1.5 mm (n=10). Sternal, lateral and ventral papillae of first thoracic segment as in Fig. 5L. Dorsum of eighth and terminal segments as in Fig. 5M.

Material examined. Holotype, male, from *Cortaderia selloana*, Zapateira, A Coruña, Spain, IX-19-2018, J. Fagúndez, deposited in USNM. Paratypes, 6 males, 8 females, 4 pupal exuviae, and 20 larvae, same data as holotype, deposited as follows: 1 male, 1female, 5 larvae in Museo Nacional de Ciencias Naturales, Madrid, Spain; 1 male, 1 female, 5 larvae in The Natural History Museum, London, United Kingdom; remainder in USNM.

Etymology. *Spanolepis selloanae* is named after the specific epithet of the host plant. That name originally honored Friedrich Sellow (1789-1831), a Prussian botanist and zoologist

who made extensive collections of flora and fauna in Brazil, Uruguay and Argentina from 1814 to 1831 (Rego et al. 2013).

Remarks. To initially identify the gall midge on pampas grass we needed to ascertain that it did not belong to any other of the seven Lasiopteridi previously recorded from flower heads of grasses. All are from Europe and/or North America and currently in *Dasineura*, but none of them is well known. We list them here with the specific differences that distinguish them from *S. selloanae*.

Dasineura airae (Kieffer) (1897: 300) was described from spikelets of *Deschampsia flexuosa* (L.) Trin. (originally given as *Aira flexuosa*) in France. It has not been found again since its original collection (Skuhravá et al. 2005). The original description includes no illustrations but does mention that the abdomen has transverse bands of scales, which S. *selloanae* lacks.

Dasineura alopecuri (Reuter) (1895: 3) (synonym, *Dasineura agropyronis* Barnes (1927: 214)) comes from *Alopecurus pratensis* L. and possibly *Elymus repens* (L.) Gould. It is widespread in northern Europe from where it immigrated into Canada (Ontario and New Brunswick) and New Zealand. Larvae of this species feed in seeds. The most striking distinguishing character of *D. alopecuri* is the pointed fused cerci that Reuter (1895) illustrated, as did later Sylvén and Tastás-Duque (1993). Barnes (1930) reported that Reuter's specimens had banded scales on the abdomen and he later (Barnes 1946) stated that the larva had a spatula. All three attributes separate this species from *S. selloana*. Barnes's *D. agropyronis* was based on females found swarming in large numbers on *E. repens* (as *Agropyron repens*). Barnes (1930) sank *D. agropyronis* under *D. alopecuri*, writing only that "it is undoubtedly *D. alopecuri*." Nevertheless, the species should eventually be reexamined if only because it was found associated with a different host.

371 *Dasineura dactylidis* Metcalfe (1933: 329) is widespread in Europe on *Dactylis glomerata*
372 L. In England adults were found mating at the end of May. Larvae fed on seeds and were
373 full-grown by end of June. One generation occurred per year. Metcalfe (1933) noted that
374 the adult abdomen had “dorsal bands of dark fuscous scales" and that the "harpes (the
375 gonocoxal mediobasal lobes) [were] irregularly digitiform." Both characters differentiate
376 this species from *S. selloanae*.
377 *Dasineura festucae* Barnes (1939: 172) is widespread in Europe on *Festuca rubra* L. Adults
378 were reared in June from seed kept over the winter. Duncan Sivell (in litt.) kindly sent a
379 photo of the abdomen of one of the syntypes that shows rows of wide scales on the terga,
380 which the new species lacks.
381 *Dasineura graminis* Felt (1908: 342) was originally collected in New York (USA) from
382 *Agrostis capillaris* L. (originally as *Agrostis vulgaris*). It is based on two females caught in
383 early June while laying eggs on flower heads. Their abdominal terga are well covered with
384 wide scales. It is possible that *D. graminis* is an introduced species. A *Dasineura* identified
385 by Barnes (1931) as this species was reared from seeds of the same host in England. Larvae
386 of that record fed on the developing seeds and the species reportedly has at least two
387 generations per year (Barnes 1946).
388 *Dasineura poae* Mühle (1957: 547) is from northern Europe on *Poa pratensis* L. Larvae
389 develop in the inflorescence. The female of this species was described with pointed fused
390 cerci and the larva with a spatula and six terminal papillae. All three attributes distinguish
391 this species from S. *selloanae*.
392 *Dasineura trisetae* Barnes (1939: 174) is known from Austria from *Trisetum flavescens*
393 (L). P. Beauv. Barnes separated the male of this species from *D. festucae* by its male
394 “ventral lamella” (hypoproct) “that … had a shallow but wide emargination, each lobe
395 approaching equilateral triangle in shape, roundly pointed,” as opposed to that of *D.*

396 *festucae* “with a deep V-shaped emargination, each lobe narrow, roundly pointed.” In a
397 companion paper on *D. trisetae*, Watzl (1939) treated the biology and all stages and
398 included some fine illustrations. Larvae of this species feed in the seed, have a spatula, and
399 form a cocoon after they drop to the soil. In contrast, larvae of *S. selloanae* lack a spatula
400 and remain in the plant to pupate.
401
402 **Discussion**
403 The new species of gall midge is placed in a new genus because of its distinctiveness
404 compared to all other known Dasineurini. We suspect that the new species is native to South
405 America and was probably introduced into Europe with its host plant. This is supported by
406 several exclusive morphological traits not found in European Dasineurini. No Dasineurini
407 or Lasiopteridi has been described from Poaceae in South America (Gagné 1994). Further
408 field work and collections are needed to establish the origin of *S. selloanae*.
409 From the DNA results, we confirmed that the larvae and adults found in *C. selloana* belong
410 to the same species. Other studies have successfully applied DNA genotyping to the
411 identification of larval specimens of Diptera (Failla et al. 2016). It is interesting that none
412 of the 449 COI sequences available in Genbank for genus *Dasineura* are a close match to
413 any of the two haplotypes of *S. selloanae*. Closer matches were observed for *J. glechomae*
414 and *Macrolabis fagicola* but these feed on Lamiaceae and Fagaceae, respectively. The two
415 species belong to genera that are closely allied to *Dasineura* and differ in the same ways as
416 *Dasineura* to *Spanolepis* (Gagné 2018). Our phylogenetic results rule out a close
417 relationship with any of the other genera included in the study. Disentangling the
418 evolutionary history of the tribe Dasineurini, and therefore a proper placing of the genus
419 *Spanolepis*, requires a much denser taxon and gene sampling which is beyond the scope of
420 the present work.

421

422 *Spanolepis selloanae effects on the host plant*

423 In our study area, we found *S. selloanae* only on female plants, none in hermaphrodite
424 plants at any stage. We did not find any viable seeds in hermaphrodites in the studied
425 population, where we recorded a sex ratio of 1:1.25 in favour of hermaphrodite plants.
426 According to the literature, female plants develop exclusively unisexual flowers, but
427 hermaphrodite plants produce fertile anthers and viable seeds, albeit at lower fertility rates
428 than female plants (Connor 1973, 1981, Astegiano 1995, Lambrinos 2001). Connor
429 (1973, 1981) found that female plants produce larger seeds than hermaphrodites, probably
430 related to a higher seed viability and seedling vigour. Seed viability was 99% in female
431 plants, and 47% in hermaphrodite plants on populations from New Zealand (Knowles and
432 Ecroyd 1985). In addition, sex ratio is commonly described as 1:1 (Connor 1973). Our
433 findings, which show different results for hermaphroditism and sex ratio, has clear
434 implications on the impact of the midge and its effects on the population.
435 The new gall midge was observed to reduce seed production to nearly one fourth,
436 considering the ratio of larvae and intact seeds in the population. This can be considered
437 a high prevalence compared to other similar species. For example, Ahee et al. (2013)
438 found a mean prevalence of 9.5% of *Stenodiplosis phragmicola* Sinclair and Ahee, a gall
439 midge of the supertribe Cecidomyiidi that feeds on seeds of *Phragmites australis*. In our
440 population, viability of the remaining intact seeds was not affected.

441

442 **Conclusions and applications**

443 *Spanolepis selloanae* is, to the best of our knowledge, the only described natural enemy
444 of *C. selloana*, a harmful invader of the coasts of southern Europe. The larvae strongly
445 affect seed production, the main source of propagation of *C. selloana*. The insect is

446 therefore a good candidate for biological control of the invasive plant. The description of
447 the new species and its effects on the host plant is a first step towards the development of
448 a new control agent for the weed, which should follow the standard procedures of
449 assessing its life cycle, native range, potential impacts on non-target species, and
450 feasibility of translocation to other populations of *C. selloana* in the Iberian Peninsula
451 and elsewhere.
452
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630 **Figure Legends**

631

632 **Fig. 1.** A. Location of the study site (yellow star) in A Coruña council (pink borders)
633 located in NW Galicia (B), in NW Spain (C). Urban areas are in red, rural areas in green,
634 and semi-urban areas in orange. D shows sampling in the studied population, E is a detail
635 of the inflorescence of one female plant.

636

637 **Fig. 2.** Reproductive phenology of female plants of *C. selloana* (A) and phenology of *S.*
638 *selloanae* (B).

639

640 **Fig. 3.** Bayesian phylogram obtained for 42 members of the tribe Dasineurini using
641 *Asteromyia carbonifera* and *Lasioptera arundinis* as outgroups. The position of
642 *Spanolepis selloanae* is highlighted. Support values (Bayesian posterior probabilities /
643 ML > 50% bootstrap / MP > 50% bootstrap) are shown next to nodes, above branches.

644

645 **Fig. 4.** *Spanolepis selloanae*. A, Female head. B, Wing. C, Larva. Scale line = 0.1 mm.

646

647 **Fig. 5.** *Spanolepis selloanae*. A, Male third flagellomere (ventral) B, Female third
648 flagellomere (ventral). C, Acromere. D, Male sixth through eighth tergites (dorsolateral).
649 E, Gonopod, cerci and hypoproct (dorsal). F, Gonostylus (ventral). G, Gonocoxal
650 mediobasal lobes and aedeagus (dorsal). H, Male sixth through eighth sternites (ventral).
651 I, Female fused cerci and hypoproct (dorsolateral). J, Female sixth tergite through fused
652 cerci (dorsal). K, Anterior structures of pupal exuviae. L, Larva, ventral setation of
653 prothorax: from center, sternal papilla, triplet of lateral papillae and ventral papilla. M,
654 Eighth and terminal segments (dorsal).

655

Figure 1

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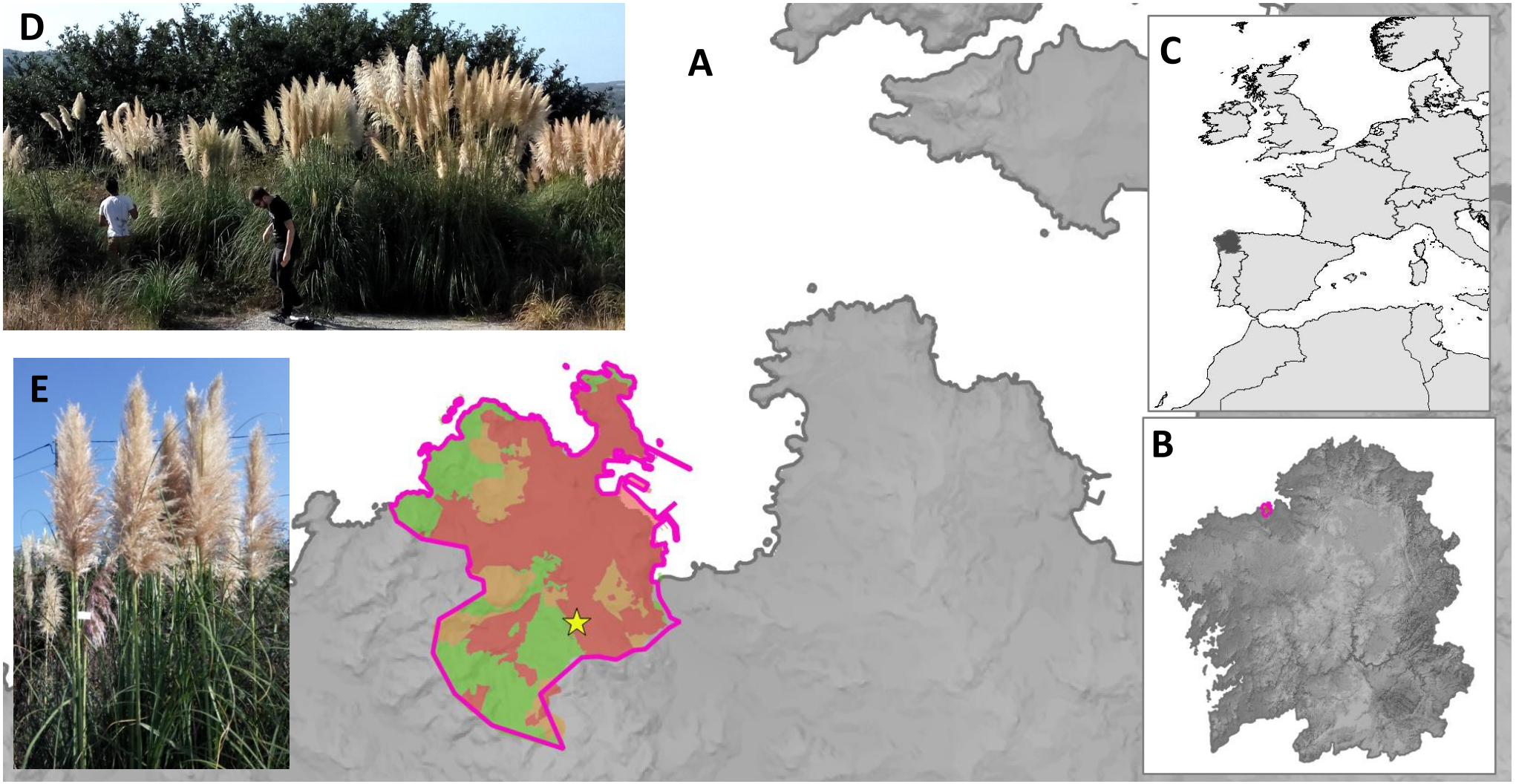

Figure 2

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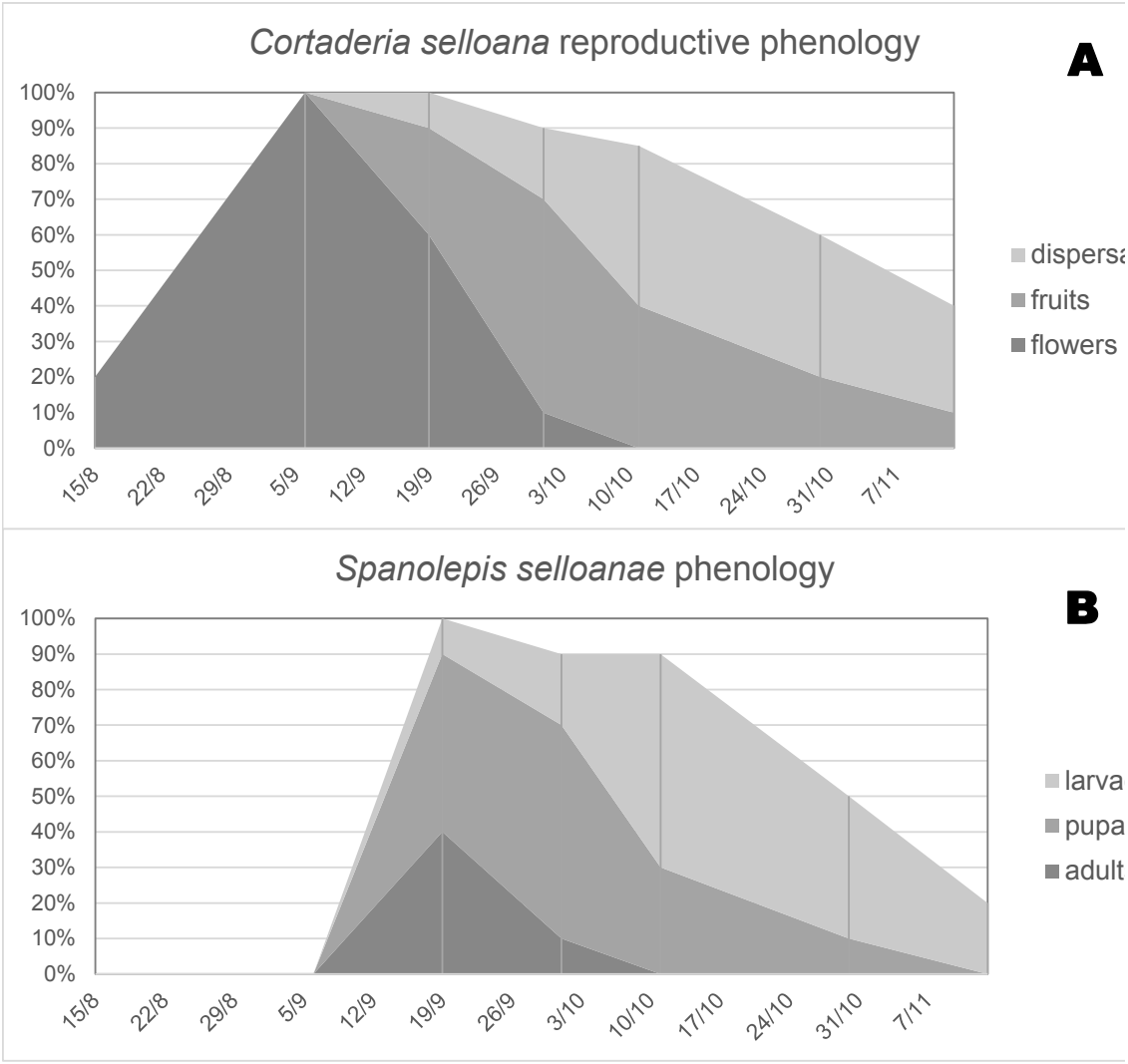

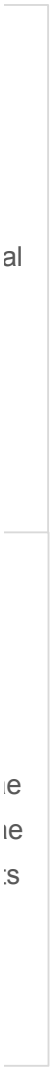
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	15-Aug	6-Sep	19-Sep	1-Oct	11-Oct	30-Oct
flowers	0.2	1	0.6	0.1	0	0
fruits	0	0	0.3	0.6	0.4	0.2
dispersal	0	0	0.1	0.2	0.45	0.4

	15-Aug	6-Sep	19-Sep	1-Oct	11-Oct	30-Oct
adults	0	0	0.4	0.1	0	0
pupae	0	0	0.5	0.6	0.3	0.1
larvae	0	0	0.1	0.2	0.6	0.4

13-Nov
0
0.1
0.3

13-Nov
0
0
0.2

Figure 3

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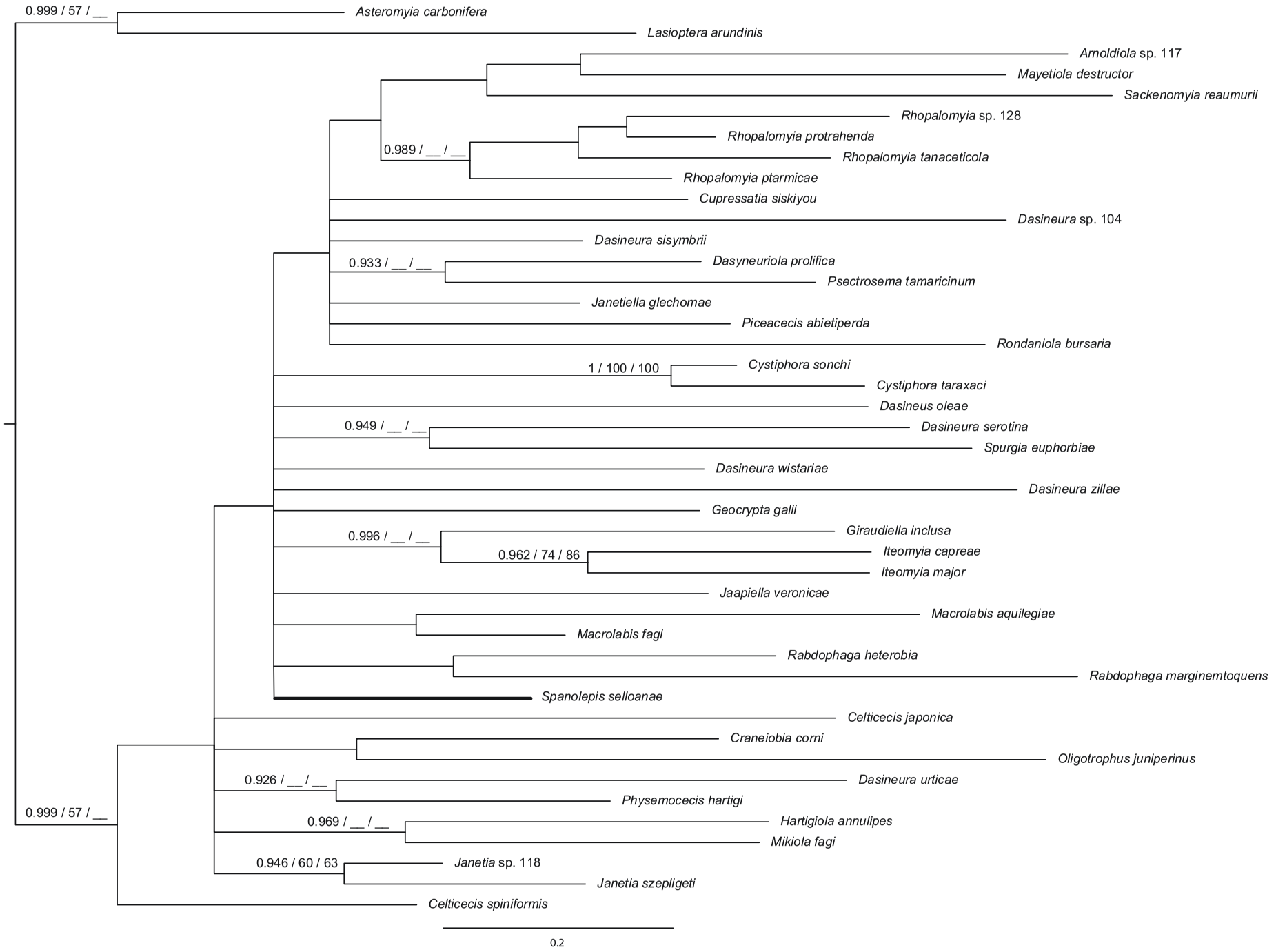

Figure 4

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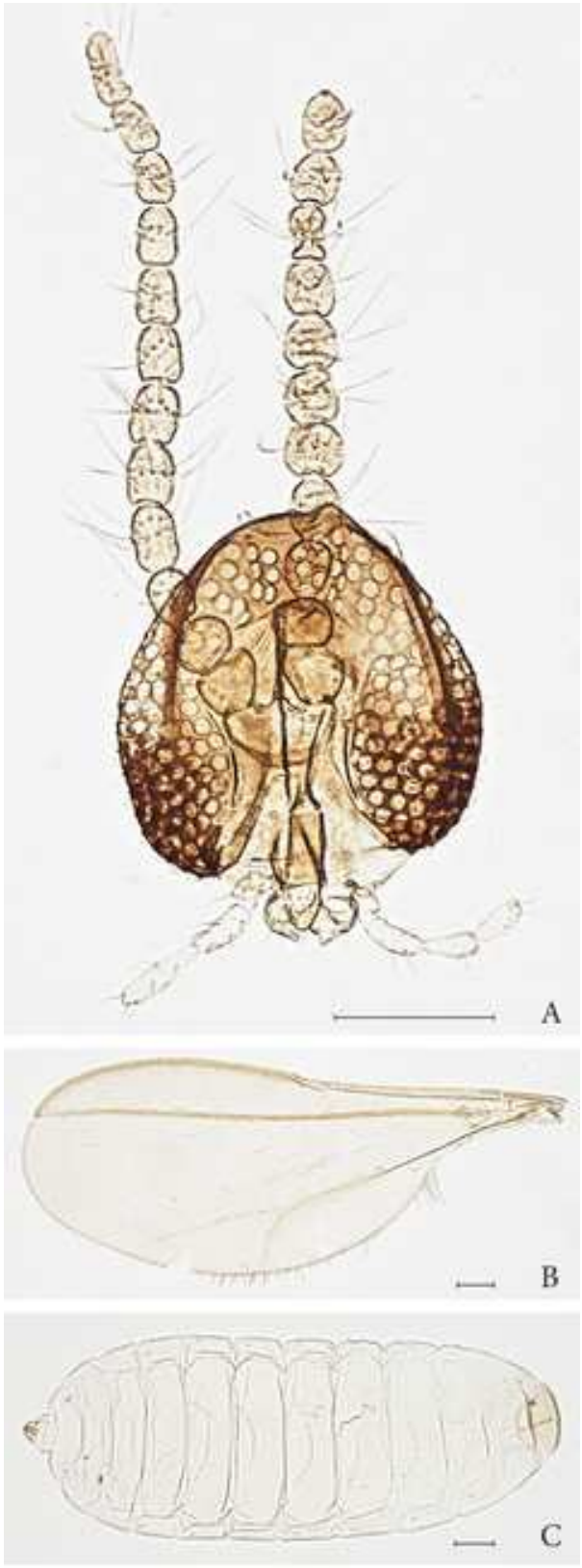

Figure 5

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