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New records document *Cystiphora sonchi* (Vallot) (Diptera: Cecidomyiidae) and associated parasitoids (Hymenoptera) in the continental United States

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New records document *Cystiphora sonchi* (Vallot) (Diptera: Cecidomyiidae) and associated parasitoids (Hymenoptera) in the continental United States

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Abstract. A gall midge, *Cystiphora sonchi* (Vallot, 1827) (Diptera: Cecidomyiidae), is reported for the first time in the continental United States of America from the states of Minnesota, North Dakota, and South Dakota. The gall midge is an obligate parasite of *Sonchus* L., including perennial sowthistle, *Sonchus arvensis* L., a weed that was the impetus for earlier releases of *C. sonchi* as a biological control in Canada. Patches of *S. arvensis* were commonly infested with *C. sonchi*, and often leaves were densely galled. Dissections of galled leaves led to the novel finding of multiple *C. sonchi* larvae in some individual galls. In addition, three parasitoids emerged from galls sampled in South Dakota: *Aprostocetus* cf. *atticus* Graham, *Ceraphron* sp., and a possible new species of *Lyrcus* Walker. Further research is warranted to determine the geographic extent of *C. sonchi* and its parasitoids in the USA, and to determine the impact of *C. sonchi* on its weedy hosts.

Key words. Pustule galls, gall midge, geographic range extension, biocontrol, *Aprostocetus*, *Lyrcus*. **ZooBank registration.** urn:lsid:zoobank.org:pub:9B8804E1-002B-45F4-8C62-82E77F876D45

Introduction

Cystiphora sonchi (Vallot, 1827) (Diptera: Cecidomyiidae) is a gall midge native to Eurasia that uses host plants, particularly within the sowthistle genus Sonchus L. (Compositae) (Gagné 1989; Bayram et al. 2005). Its host plants include S. arvensis L. (perennial, or field, sowthistle) and S. oleraceus L. (annual, or common, sowthistle) (Gagné 2010; Ben Halima Kamel et al. 2019). Female C. sonchi oviposit through stomata on the underside of leaves (DeClerck and Steeves 1988). Upon hatching, larvae feed on leaf mesophyll, induce small circular pustule galls, and develop through three instars (Peschken et al. 1989). Cystiphora sonchi typically has two to three generations per year (Gagné 1989; Peschken et al. 1989; Bayram et al. 2005). Pupation occurs within the leaf galls of the first two generations, and mature larvae of the third generation drop from the leaf and overwinter in the soil (Peschken et al. 1989). Adults emerge from the soil the following spring (Peschken et al. 1989).

Sonchus arvensis and S. oleraceus are native to Europe and western Asia (McWilliams 2004; Bryson and DeFelice 2010) and both established in east-central North America in the late 19th century (McWilliams 2004). They are now naturalized and distributed throughout most of North America north of Mexico (USDA Plants 2020). Sonchus arvensis and S. oleraceus are particularly common in disturbed areas (Bryson and DeFelice 2010). Sonchus arvensis is designated as a noxious weed in several states (McWilliams 2004).

Cystiphora sonchi was released in the 1980s in Canada for biological control of perennial sowthistle and subsequently established in the provinces of Alberta, Saskatchewan, Manitoba, Nova Scotia and possibly New Brunswick (McClay and Peschken 2002). Surveys for C. sonchi in Canada (McClay and Peschken 2002; Peschken 2013) found that it was used by various parasitoids, including a larval endoparasitoid, Aprostocetus cf. atticus Graham (Hymenoptera: Eulophidae), Neochrysocharis formosus (Girault) (Hymenoptera: Eulophidae)

and *Chrysonotomyia* sp. (Hymenoptera: Eulophidae), and a *Zatropis* Crawford species (now *Lyrcus* Walker; Hymenoptera: Pteromalidae). *Aprostocetus atticus* Graham was reared from a questionably identified species of *Cystiphora* Kieffer on *Chondrilla* sp. (Asteraceae) from Greece (Graham 1987). Two additional species of *Aprostocetus* Westwood, *A. eleuchia* (Walker, 1839) and *A. microscopicus* Rondani, 1877, were previously reported as parasitoids of *C. sonchi*. *Aprostocetus eleuchia* was initially reported from *C. sonchi* by Domenichini (1966a), but Graham (1987) later indicated that the host identification needed confirmation. *Aprostocetus microscopicus* was reported from *C. sonchi* in the original description (Rondani 1877), and Graham (1987) indicated hosts reported from countries other than Italy by Domenichini (1966a, b) are incorrect. However, no published scientific reports have been found regarding insects associated with *C. sonchi* pustule galls on *Sonchus arvensis* in the USA.

The objective of this paper is to report on the first observations of *C. sonchi* and its parasitoids in the continental USA.

Materials and Methods

Leaves of *S. arvensis* with pustule galls were first noticed 0.6 km north of Brookings, South Dakota, USA, and Nisswa, Minnesota, USA in early June 2018. Galled leaves were haphazardly sampled over the following several weeks, placed into small, transparent plastic bags or cylindrical containers, and transported to the USDA-ARS North Central Agricultural Research Laboratory (NCARL), Brookings, SD. The leaves were held in the laboratory at room temperature and monitored daily for insect emergence. Insects that emerged were collected with a paintbrush and placed into a glass vial containing 70% ethanol. Specific locations and dates of leaf collection were recorded. Representative samples of adult insects that emerged were shipped in vials of alcohol to the USDA-ARS Systematic Entomology Laboratory (SEL), Washington, D.C. for identification and for voucher deposition. Adult gall midges were identified using keys in Gagné (1989) and comparison with known specimens and confirmed as *C. sonchi* by Dr. Raymond Gagné. Galls from leaves of *S. arvensis* collected in 2018 near Brookings were dissected and examined in the laboratory to obtain information on immature stages of insects within them.

Ethanol-preserved parasitoid specimens were dehydrated through increasing concentrations of ethanol and transferred to hexamethyldisilazane (Heraty and Hawks 1998) before card-mounting. Specimens were determined to genus by the second author (MWG) via sight identification or using Gibson et al. (1997). Specimens were then identified through species level keys: an unpublished key to North American *Lyrcus* species provided by Gary Gibson (Agriculture and Agri-Food Canada, retired) and a key to American (Burks 1967) and European *Aprostocetus* (Graham 1987). The ceraphronid was identified by M. Buffington using Dessart and Cancemi (1987).

Abbreviations for museums mentioned include: NMHUK, Natural History Museum, London, UK; USNM, United States National Museum of Natural History, Washington, D.C., USA. All reared specimens are deposited at USNM.

The discovery of pustule galls at the Brookings and Nisswa sites prompted sampling at other sites in South Dakota and Minnesota in 2018 and in North Dakota in 2019. Sowthistle plants with pustule galls at other sites were either photo-documented or leaves of infested plants were sampled by placing them in plastic bags and transported in a cooler to NCARL, where they were held in the laboratory for emergence of adult insects.

Samples of *Sonchus arvensis* were identified using descriptions in Bryson and DeFelice (2010) and USDA Plants (2020). In some cases, plants in the early pre-reproductive stage were identifiable only to *Sonchus* sp. because of morphological similarity among congeneric species at young development stages.

In addition to field sampling, we checked for any previous records of *C. sonchi* in the USA by consulting the online database of the Symbiota Collections of Arthropods Network (SCAN 2020) and by querying various internet search engines for records of *C. sonchi* in the USA.

Results

Two types of adult insects emerged from galled sowthistle leaves, namely a gall midge (Diptera: Cecidomyiidae) and two morphospecies of parasitoid wasps (Hymenoptera: Eulophidae, Pteromalidae). Each set of insects is discussed separately below.

Cystiphora sonchi (Vallot, 1827) (Diptera: Cecidomyiidae)

Gall midges that emerged from pustule galls on sowthistle leaves from Brookings, South Dakota, and Nisswa, Minnesota, were identified as *C. sonchi*, and these specimens were vouchered at the SEL. Additional specimens of *C. sonchi* obtained from the other sampling sites in 2018 and 2019 were vouchered in the NCARL insect collection. Based on these various samples, new state records of *C. sonchi* are presented here from Minnesota, North Dakota, and South Dakota (Fig. 1).

South Dakota (**new state record**), 1 km N of Brookings, Brookings County, N 44°20.406′, W 96°47.335′, 10-VII-2018, Ex. *Sonchus arvensis*, coll. Eric Beckendorf. USA, South Dakota, Brookings County, Opland Loop Road, 1 km S of Brookings, 9-VII-2018, Ex. *Sonchus arvensis*, coll. Eric Beckendorf. USA, South Dakota, Brookings County, 1.5 mi N Brookings, Eastern South Dakota Soil and Water Research Farm, N 44°19′, W 96°46′, 16-VII-2018, Ex. *Sonchus arvensis*, coll. Louis Hesler.

Minnesota (**new state record**), Crow Wing County, Nisswa, N 46.5416633°, W 94.3492720°, 13-VII-2018, Ex. *Sonchus* sp., coll. Sophia Conzemius. USA, Minnesota, Dakota County, Eagan, N 44.824245, W 93.170886, 15-VII-2018, Ex. *Sonchus arvensis*, coll. Sophia Conzemius.

North Dakota (**new state record**), Tomahawk National Wildlife Refuge, 2 km NW of Rogers, Barnes County, N 47°04′54.6″, W 98°11′31.9″, 24-VI-2019, x. *Sonchus* sp., colls. Eric Beckendorf and Janna Julius.

Sowthistles with pustule galls of *C. sonchi* were common in the areas sampled. Additional observations of leaves of *Sonchus* spp. with pustule galls consistent with infestation by *C. sonchi* were observed (but no specimens collected) at the following locations: USA, North Dakota, Steele County, North Golden Lake, 7 km SW of Hatton, N 47°35′08.7″, W 97°37′40.6″, 24-VI-2019. USA, Minnesota, Koochiching County, International Falls, 16-VIII-2019.

Galled sowthistles varied widely in the degree to which leaves were galled, with moderate to heavily galled leaves common (Fig. 2), including an instance of one leaf with at least 201 pustule galls (Fig. 2C). Dissections of individual pustule galls from the Brookings area show that they typically contained a single *C. sonchi* larva or parasitoid larva, but occasional dissections revealed multiple *C. sonchi* larvae per gall (Fig. 3). Galls with multiple parasitoid larvae were not found.



Figure 1. Distribution map of *Cystiphora sonchi* in North America. Pink indicates Canadian provinces with previous records of *C. sonchi* and orange indicates states with new records in the USA.

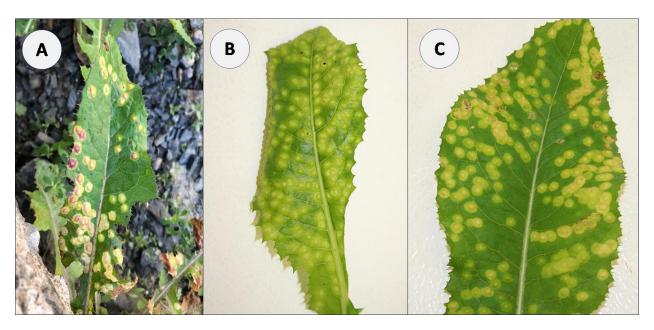


Figure 2. Sowthistle leaves with pustule galls of *Cystiphora sonchi*. **A)** International Falls, Minnesota, USA, in 2019. **B–C)** Heavily galled leaves near Brookings, South Dakota, USA, in 2018.



Figure 3. Individual pustule galls on Sonchus arvensis with variable numbers of larval Cystiphora sonchi per gall.

Online records of *C. sonchi*. No USA records of *C. sonchi* were found in the SCAN database. However, digital images of blister galls on *S. arvensis* were found online for two locations in the USA. One was from Grand Forks, Grand Forks County, North Dakota, on 5 August 2015 submitted by Carl D. Barrentine, with images showing galls on sowthistle leaves consistent with those made by *C. sonchi* (https://bugguide.net/node/view/1116608/bgimage). A second image consistent with *C. sonchi*-galled sowthistle leaves was from Le Sueur, Le Sueur County, Minnesota, N 44.4883346558, W 93.8752746582, 2019-08-10 (https://www.discoverlife.org/mp/20l?id=INAT31078790). These reports corroborate our discoveries in other locations in North Dakota and Minnesota.

Recovery of parasitoids from pustule galls of sowthistle leaves. Parasitoid wasps emerged from galled leaves of *S. arvensis* from: USA, South Dakota, 1 mi N Brookings, Brookings County, N 44°20.406′, W 96°47.335′, 10-VII-2018, 11-VII-2018, ex. *Sonchus arvensis*, coll. Louis Hesler. The parasitoids belonged to three genera, *Aprostocetus* sp. (Hymenoptera: Eulophidae), *Lyrcus* sp. (Hymenoptera: Pteromalidae), and *Ceraphron* sp. (Hymenoptera: Ceraphronidae). These specimens are vouchered at the Smithsonian Institution, National Museum of Natural History.

Aprostocetus cf. atticus (Fig. 4A): both males and females key easily to A. atticus in Graham's key (1987) to European species of Aprostocetus and matches the description and illustrations. It does not key in Burks (1967). To our knowledge, this species is only known from the type series (7 males and 2 females) in the NHMUK. Hence, that species is not represented in the USNM, so we are unable to compare against authoritatively identified material. It would not be surprising if A. atticus was co-introduced with C. sonchi.

Lyrcus, possible new species (Fig. 4B): males and females were run through the incomplete, unpublished key to North American species of *Lyrcus*. Although these specimens are superficially similar to *L. justicia*, they keyed beyond that couplet to an unidentified new species. It is possible that this particular species was introduced along with *C. sonchi*.

Ceraphron sp. (Fig. 4C): a single specimen was recovered from the rearing and species in this genus are parasitoids of insects in semi-concealed situations (Diptera, Hemiptera, Neuroptera and Thysanoptera) or hyperparasitoids of Hymenoptera prepupa.

Discussion

This report contains the first collection records of *C. sonchi* and associated parasitoids in the continental USA. Although our earliest collections of *C. sonchi* occurred in 2018, the online image of galled sowthistle leaves at Grand Forks, North Dakota, indicates that *C. sonchi* had established at least 80 km into the United Sates by 2015. Peschken (2013) stated that *C. sonchi* had been observed in Minnesota but provided no details. The records of *C. sonchi* at our locations were directly south of Manitoba, one of the Canadian provinces in which this gall midge is known to have established following its release in Canada for biological control of perennial sowthistle (McClay and Peschken 2002). Accordingly, the most parsimonious explanation for the occurrence of *C. sonchi* in the USA is simply the southward geographic spread of this species from Canada.

The current range of *C. sonchi* in the Palearctic realm covers a widely varied geography that extends from Tunisia in northern Africa (Ben Halima Kamel et al. 2019) through Europe (Skuhravá and Skuhravý 1993, 1997, 2004; Skuhravá 1997, 2004; Rizzo and Massa 1998; Skuhravá et al. 2002; Bayram et al. 2005) and eastward in Asia to Kazakhstan (Fedotova 2000). In North America, its primary host plant, *S. arvensis*, is distributed throughout most of Canada and much of the continental USA (USDA Plants 2020). Thus, new records of *C. sonchi* in the USA are not surprising, given its known distribution in warmer regions of Europe, Asia and northern Africa, and the extensive geographic distribution of its host plants in North America. Assuming analogous rates of spread southward from the provinces of Alberta and Saskatchewan, additional records of *C. sonchi* are predicted at similar latitudes due west of our collection sites in North Dakota and South Dakota. Furthermore, although our records from Minnesota, North Dakota, and South Dakota document a southward range extension of *C. sonchi*, we do not know if they necessarily reflect the southern limit of its range, and additional sampling at locations farther south is encouraged.

Cystiphora sonchi was well established at sampling sites in the USA, based on the relative ease at which we were able to locate galled sowthistle plants and the high density of galls on many of the leaves. Although we did

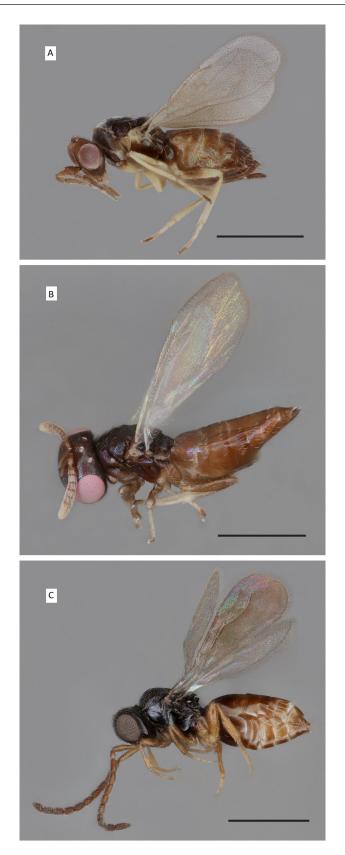


Figure 4. Hymenoptera associated with pustule galls of *Cystiphora sonchi*. **A)** *Aprostocetus* cf. *atticus*. **B)** *Lyrcus*, possible new species. **C)** *Ceraphron* sp.

not extensively quantify the density of galls, frequently the majority surface area of individual leaves of *S. arvensis* was occupied by pustule galls of *C. sonchi* (Fig. 2B, C). Zollinger and Parker (1999) reported as many as 721 galls were formed on one plant of field sowthistle. However, Lemna and Messersmith (1990) stated that no reduction in field sowthistle densities because of *C. sonchi* had yet been observed, and Peschken (2013) stated that *S. arvensis* had not been controlled in Canada. The impact of galling by *C. sonchi* on *S. arvensis* was not within the scope of our study but may be a relevant topic to address in future research.

It is possible that the recovered parasitoids that occur in the native range of *C. sonchi* were accidentally introduced along with this gall midge when it was released against *S. arvensis*. It is also possible that similar native species adapted to this new host resource. Our parasitoid identifications, the *Aprostocetus* and *Lyrcus*, very closely match two of those reported by McClay and Peschken (2002) as a result of their survey. It is beyond the scope of this paper to provide differential diagnostics for North American species of *Lyrcus* and *Aprostocetus*; there are needs for revisions of both genera for the North American fauna.

The parasitoids that we collected from pustule galls belonged to species in two genera from two families, *Aprostocetus* and *Lyrcus*. At least one species of *Aprostocetus* (identified as "sp. near *atticus*") is known to parasitize larval *C. sonchi* in Canada (McClay and Peschken 2002). *Lyrcus* spp. are known in the USA (Gibson 2013), but we did not find any record of *C. sonchi* as a host although they are often recovered as associates of gall inducing insects. McClay and Peschken (2002) reported that at least three other species of parasitoids attack *C. sonchi* in Canada. Further research is needed on the specific identity, prevalence and impact of parasitoids of *C. sonchi* in the USA.

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