Rhinusa asellus (Gravenhorst) (Coleoptera: Curculion Eurasian Weevil New to North America, with a Summa Other Adventive Rhinusa in North America and a Key Species.

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RHINUSA ASELLUS (GRAVENHORST) (COLEOPTERA: CURCULIONIDAE), A EURASIAN WEEVIL NEW TO NORTH AMERICA, WITH A SUMMARY OF OTHER ADVENTIVE *RHINUSA* IN NORTH AMERICA AND A KEY TO SPECIES.

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Abstract.—The discovery of *Rhinusa asellus* (Gravenhorst), a Palearctic weevil which breeds in stems of *Verbascum* spp. (Scrophulariaceae), in North America is documented for the first time. This species is currently known from five northeastern states (Maine, Massachusetts, New Hampshire, New York and Vermont). It was first identified in a funnel trap sample from Albany Co., New York collected in May 2017. A specimen was then discovered in unidentified bycatch material from Worcester County, Massachusetts collected in July 2013. In addition to a morphological identification, DNA barcoding of the mtCOI gene confirmed the conspecificity of the North American specimens with specimens from Germany. There are now five species of *Rhinusa* known to occur in North America: *R. antirrhini* (Paykull), *R. asellus* (Gravenhorst), *R. linariae* (Panzer), *R. neta* (Germar), and *R. tetra* (Fabricius). We give a summary of distribution and biology, and provide an updated key to all species of *Rhinusa* known to occur in North America.

Key Words: Exotic species, new records, European mullein weevil, Scrophulariaceae, northeastern United States

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Weevils (Curculionoidea) represent one of the most diverse groups of beetles in the world. Roughly 62,000 described species comprise about 15.5% of the approximately 400,000 species of beetles currently described worldwide (Oberprieler et al. 2007, 2014). Their phytophagous nature, coupled with a global economy, has facilitated the unintentional introduction of several nonnative species into North America in recent years (Anderson et al. 2013, 2018; Anderson and Korotyaev 2004; Hoebeke and Spichiger 2016; O'Brien 2000; Sweeney et al. 2012). Historically, a few adventive weevils have become significant agronomic crop and forest pests. Many non-native weevils have also been introduced intentionally for the purpose of biocontrol of invasive plants.

The genus *Rhinusa* Stephens (formerly treated as a subgenus of *Gymnetron*

Schoenherr) is comprised of approximately 50 named Palearctic species (Alonso-Zarazaga et al. 2017, Caldara et al. 2010). Heretofore, the North American fauna has included four Rhinusa species (O'Brien and Wibmer 1982, De Clerck-Floate and Harris 2002), all adventive to this region, with scattered records known from across much of the United States and Canada. All known species of Rhinusa utilize Plantaginaceae (Linaria Mill., Antirrhinum L., Misopates Raf., Chaenorhinum (DC.) Rchb. and Kickxia Dumort.) and Scrophulariaceae (Verbascum L., Scrophularia L.) - both families placed together in the order Lamiales (APG 2016) - as host plants (Caldara et al. 2010, Gassmann et al. 2014, Marquess 2000), but specializing mostly on a few closely related host species. Recently, a fifth non-native species, Rhinusa asellus (Gravenhorst), has been discovered in the northeastern United States. We document this discovery herein, give a summary of the other adventive species of Rhinusa in North America, and provide an updated key to all species of Rhinusa known to occur in North America.

On 26 May 2017, an unrecognized weevil was collected from a multiple funnel trap baited with alpha-pinene, ethanol, ipsenol and ipsdienol by New York State Department of Environmental Conservation Forest Health staff in the Albany Pine Bush Preserve in Albany (Albany Co.), New York. This specimen was sent to MFD for species determination in January 2018. At that time, an identification was not made, however images were taken of the dorsal and lateral habitus. In February 2018, after revisiting this specimen, MFD reviewed keys (Caldara 2014) to European species of Rhinusa and concluded that R. asellus may be a possible candidate for the species of the unknown weevil. Images were emailed to RC and a tentative determination, based on the morphology observed in these images, was made as a female of R. asellus. This prompted MFD to examine unidentified Curculionidae specimens in his personal collection. A specimen was found, collected as bycatch from a previous study in Massachusetts on 3 July 2013 (Dodds et al. 2016), that matched the external characters of R. asellus. Images of this specimen were emailed to RC, who again made a determination of a female of R. asellus. Images were also posted by MFD to the website BugGuide (https://bugguide.net/node/view/1483420 https://bugguide.net/node/view/ and 1518571). Both specimens were then sent to the USDA-ARS Systematic Entomology Laboratory (United States National Museum of Natural History, Smithsonian Institution, Washington, DC) and final determinations of both specimens were confirmed as R. asellus. These represent the earliest confirmed records of R. asellus in North America. Subsequently, a total of 177 additional specimens were hand-collected during informal surveys of Verbascum thapsus L. in June–December 2018 from Maine, Massachusetts, New Hampshire and Vermont. Based on the distribution of these specimens collected over a span of five years, it is assumed that R. asellus is well established in the northeastern United States.

MATERIALS AND METHODS

Images used in this paper (except those of *R. neta*) were created by MFD using a Leica M165C stereoscope outfitted with a 1.0x Leica achromatic objective lens, Leica DFC420 five megapixel digital camera attachment, and automated focusing step rail controlled by Leica Application Suite Version 4.5

[Leica Microsystems (Switzerland) Ltd., Heerbrugg. Switzerland, 2014]. Up to 500 digital images were captured at different focal lengths. These images were combined into a single composite image and edited using Adobe Photoshop CC Version 19.1.5 (Adobe Systems, Inc., 2018, San Jose, California).

Identity of *R. asellus* was confirmed by Lourdes Chamorro (U.S. Department of Agriculture (USDA), Agricultural Research Service (ARS), Systematic Entomology Laboratory, United States National Museum of Natural History, Smithsonian Institution, Washington DC) using European keys (Caldara 2014) and comparing the New York and Massachusetts specimens with identified research specimens housed in the United States National Museum of Natural History, Smithsonian Institution, Washington, D.C.

Specimens collected from stems of *Verbascum thapsus* in Keene (Cheshire Co.), New Hampshire were killed directly in 95% ethanol and sequenced for mtCOI gene by Ivo Toševski (Institute for Plant Protection and Environment, Zemun, Serbia).

Voucher specimens of *R. asellus* are deposited in the University of Georgia Collection of Arthropods (Athens, GA; UGCA); the U.S. Forest Service, Durham Field Office Forest Insect Collection (Durham, NH); the University of New Hampshire Insect Collection (Durham, NH; UNHC); and the United States National Museum of Natural History, Smithsonian Institution (Washington, DC; USNM).

RESULTS AND DISCUSSION

Rhinusa asellus (Gravenhorst, 1807) (Figs. 1–2, 7–8, 13–14, 19–20)

Rhynchaenus asellus Gravenhorst, 1807: 203 (original combination). Subsequently

treated in literature as *Gymnetron asellus*, *Gymnetron (Rhinusa) asellus* and only recently as *Rhinusa asellus*. For detailed references and synonymies see Caldara (2014) and Alonso-Zarazaga et al. (2017).

Diagnosis.—*Rhinusa asellus* can be distinguished from other members of the genus in North America by the combination of its larger body size (4.0–5.6 mm), the long, nearly straight (in profile), cylindrical rostrum of the female (Fig. 7), and the location of larval development within the host (stems of *Verbascum* spp.).

Redescription.-Male: Body oval, stout. Integument black except antennae and tarsi brown. Rostrum (Figs. 8, 14) 1.10-1.20x as long as pronotum; in lateral view very slightly narrowed from base to apex; in dorsal view with subparallel sides from base to apex. Pronotum distinctly transverse (1.40–1.50x as wide as long). Elytra moderately elongate (1.25–1.35x as long as wide), suboval; interstriae clearly visible between subrecumbent to suberect, moderately dense, grayish, seta-like scales which are long, ruffled and arranged irregularly. Legs stout; femora distinctly swollen, with large tooth; protibiae distinctly curved inside near apex (Fig. 20). Protibiae (Fig. 20) and mesotibiae with distinct premucro, metatibiae lacking premucro. Female: As in male except rostrum (Figs. 7, 13) longer (1.68-1.76x as long as pronotum), subcylindrical; in lateral view slightly curved in apical third; in dorsal view sides slightly narrowing in basal third but cylindrical from antennal insertion to apex. Femora with very small tooth; protibiae straight to apex (Fig. 19). Protibiae (Fig. 19) and mesotibiae with larger premucro.

Remarks.—Among the Palaearctic species adventive in North America, *R*.



Figs. 1–6. Dorsal habitus of *Rhinusa* spp. known to occur in North America. 1, *R. asellus*, female. 2, *R. asellus*, male. 3, *R. tetra*. 4, *R. neta*. 5, *R. antirrhini*. 6, *R. linariae*; scale line = 1 mm.

asellus is more closely related morphologically and biologically to *R. tetra* (Fabricius) (Caldara 2014). Phylogenetically, these species form an apparently monophyletic clade with all other species feeding on Scrophulariaceae and well separated from all the species living on Plantaginaceae (Caldara et al. 2010).

KEY TO NORTH AMERICAN SPECIES OF *RHINUSA*

1. Protibiae and mesotibiae without premucro (Fig. 21); protibiae in male not arcuate in apical



Figs. 7–12. Lateral aspect of rostrum of *Rhinusa* spp. known to occur in North America. 7, *R. asellus*, female. 8, *R. asellus*, male. 9, *R. tetra*, female. 10, *R. neta*, female. 11, *R. antirrhini*, female. 12, *R. linariae*, female; scale line = 1 mm.

- Rostrum strongly curved at base in profile in both sexes (Fig. 12); elytra only with appressed whitish-gray setae, lacking any erect setae; tibiae with outer margin distinctly curved outwards near apex; body length 2.5–3.5 mm *R. linariae* (Panzer)

- Rostrum slender, longer (in male 0.74–0.78x and in female 0.87–0.93x as long as pronotum), in dorsal view with slightly visible scrobes and parallel-sided from antennal insertion to apex (Fig. 16), in lateral view almost of same width from base to apex, distinctly cylindrical in female; pronotum distinctly transverse (1.48–1.58x as wide as long); elytra broader, with moderately rounded sides; body length 2.3–3.2 mm
- - gradually but distinctly tapering from base to apex, in female subconical and shorter, 0.90–1.15x as long as pronotum (Figs. 9, 15);

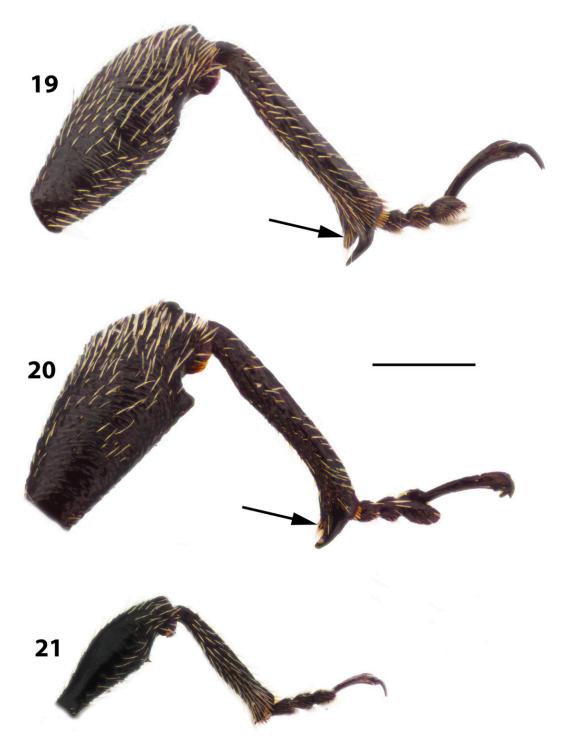


Figs. 13–18. Dorsal aspect of rostrum of *Rhinusa* spp. known to occur in North America. 13, *R. asellus*, female. 14, *R. asellus*, male. 15, *R. tetra*, female. 16, *R. neta*, female. 17, *R. antirrhini*, female. 18, *R. linariae*, female; scale line = 1 mm.

Global distribution.—*Rhinusa asellus* is native to the Palearctic Region and can be found in central and southern Europe, and states of the Caucasus. We report here the distribution as very recently given by Alonso-Zarazaga et al. (2017). Countries included are: Austria, Belgium, Bulgaria, Czech Republic, France, Germany, Greece, Hungary, Italy, Luxembourg, Macedonia, Moldavia, the Netherlands, Poland, Romania, Russia, Slovakia, Spain, Switzerland, and Turkey.

North American records.—Including the original detections and confirmation of *R. asellus* from Worcester County, Massachusetts in July 2013, and Albany County, New York in May 2017, we have documented the presence of *R. asellus* in 16 locations of 9 counties in the states of Maine, Massachusetts, New Hampshire, New York and Vermont. (Fig. 22); (records below under "Material examined"). It is likely that the actual distribution is wider than that reported here, as most of the records were collected during MFD's travels around the region.

Material examined (number of specimens of each sex collected in parentheses).-U.S.A.: Maine: York Co., Alfred, corner of Route 4 and Route 202, 43.470565°, -70.720190°, 6 December 2018, extracted from stem of Verbascum thapsus (620,529); Massachusetts: Franklin Co., Montague, Millers Falls Road. 42.577259°. -72.503589°, 21 June 2018, on Verbascum thapsus (1Q); Sunderland, Mount Toby Massif, 42.46591°, -72.53208°, 21 June 2018, on Verbascum thapsus (10,59); Worcester Co., Ashburnham, Paul Dunn Woodland Preserve, 42.6819°, -71.9634°,



Figs. 19–21. Prolegs, anterior face (arrows indicate premucros). 19, *R. asellus*, female. 20, *R. asellus*, male. 21, *R. antirrhini*, female; scale line = 0.5 mm.

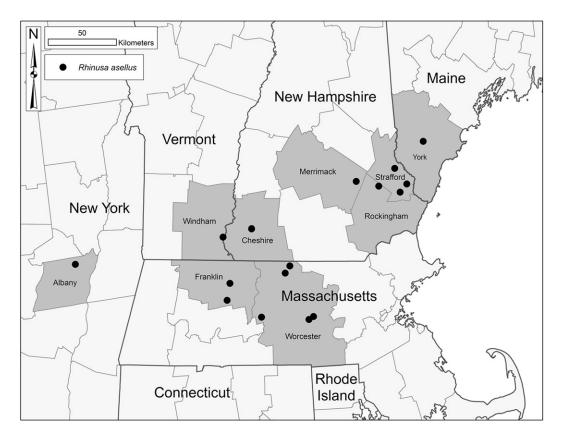


Fig. 22. Current known geographic distribution of Rhinusa asellus in North America.

3 July 2013, ex. multiple funnel trap (1Q); Hardwick, Muddy Brook Wildlife Management Area, 42.348764°, -72.228897°, 11 November 2018, extracted from stem of Verbascum thapsus (10,19); West Boylston, powerline ROW at end of Hartwell Street, 42.342786°, -71.766208°, 24 July 2018, on Verbascum thapsus (19); Winchendon, 108 Gardner Rd., 42.635508°, -72.006332°, 24 July 2018, on Verbascum thapsus (19); Worcester, 42 Maravista Road, 42.324083°, -71.807033°, 5 July 2018, on Verbascum thapsus (10). New Hampshire: Cheshire Co., Keene, Franklin Pierce Highway and West Street, 42.934221°, -72.297117°, 20 July 2018, on Verbascum thapsus (1Q); same locality, 27 Aug 2018, extracted from stem of Verbascum thapsus $(11\sigma, 12\varphi)$; Merrimack Co., Epsom, 1680 Rt. 202, 43.223889°, -71.340720°, 26 June 2018, on Verbascum thapsus (20,29); Rockingham Co., Northwood, corner of Cooper Hill Road and Route 4, 43.187322°, -71.139456°, 15 June 2018, on Verbascum thapsus (20,19). Strafford Co., Dover, corner of Arch Street and Friesian Drive, 43.193338°, -70.885700°, 20 June 2018, on Verbascum thapsus $(2\sigma, 6\varphi)$; Dur-Mast Rd., 43.141467°, ham. 271 -70.949161°, 23 July 2018, on Verbascum thapsus (19); Rochester, 84 Washington Street, 43.300411°, -70.988789°, 17 2018, on Verbascum thapsus June (20,19). New York: Albany Co., Albany Pine Bush Preserve, Madison Ave. Extension, 42.718882°, -73.884101°, 26 May 2017, ex. multiple funnel trap baited with alpha-pinene, ethanol, ipsenol, ipsdienol (19). Vermont: Windham Co.,

Brattleboro, Route 9, 160 m west of Connecticut River Bridge, 42.884337° , -72.554905°, 21 June 2018, on *Verbascum thapsus* (3σ , 5 \circ).

Host range and biology.-Larval hosts of R. asellus are restricted to species of Verbascum, commonly referred to as mullein. Documented and confirmed hosts include V. nigrum L., V. phlomoides L., V. pulverulentum Vill., V. sinuatum L., V. thapsoides Schw., V. thapsus, and V. virgatum Stokes (Caldara 2104). Halperin and Fremuth (2003) reported V. gaillardotti Boiss. as a host, however this was a misidentification of the morphologically similar R. tenuirostris (Stierlin) (Caldara 2014, Doğanlar and Üremiş 2014). Dzhanokmen (2017) reported R. asellus in fruits of Linaria pedicellata Kuprian; however, this is likely a misidentification since R. asellus is a member of the R. tetra species group which only feeds on Verbascum spp. (Caldara 2014, Caldara et al. 2010). Species groups within Rhinusa utilizing Linaria spp. as hosts include the R. antirrhini, R. linariae, R. pilosa, and R. neta groups (Caldara et al. 2010). Verbascum spp. known to occur in North America (not including formally named hybrids), all of which are introduced, include V. blattaria L., V. chaixii Vill., V. densiflorum Bertol., V. lychnitis L., V. nigrum, V. olympicum Boiss., V. phlomoides, V. phoeniceum L., V. pulverulentum, V. pyramidatum M. Bieb., V. sinuatum, V. speciosum Schrad., V. thapsus, and V. virgatum (USDA, NRCS 2018). It is possible that any of these are suitable hosts of R. asellus; however, the only species thus far found to be associated with R. asellus in North America is V. thapsus.

Biology of *R. asellus* has been well described by Gumovsky (2007). Adults can be found feeding on the shoots and leaves of the host plant beginning in late

May and early June, as the host begins to form a flower head. Oviposition sites are typically concentrated at the top portion of the host plant. These consist of openings in the plant tissue created by the female's long rostrum, into which eggs are deposited. A stain of black sap from the host plant is often found at the oviposition sites. Eggs are small (0.26–0.27mm), mushroom shaped, milky white, and develop in around 7 days. After hatching, the larva bores into the stem and feeds on plant tissues, often creating a swelling of the stem. Mature larvae will then form a pupal cell just beneath the outer layer of plant tissue within the stem. Larval development typically takes about 3 weeks, and pupae may be found on host plants starting in late June. MFD and RC have observed R. asellus to often live on the same host plant with R. tetra in North America and in Europe.

DNA analysis.—Six specimens, collected in Keene (Cheshire Co.), New Hampshire on 27 August 2018, and sequenced for the mtCOI gene shared 99.8% identity with sequenced specimens of *R. asellus* from Germany (accession number in GenBank KU908288) with only a single nucleotide difference. All of these specimens carried the same haplotype (I.Toševski in litt.).

Summary of other adventive *Rhinusa* species occurring in North America.— With the addition of *R. asellus*, five species of the genus *Rhinusa*, all of which are adventive, are known to occur in North America. Brief summaries of hosts, global distribution and biology of the other four species are as follows.

Rhinusa antirrhini (Paykull, 1800)

(Figs. 5, 11, 17, 21)

Hosts.—Mostly toadflaxes (*Linaria* spp.) and *Antirrhinum* spp.

Distribution.—*Palearctic*: Europe from United Kingdom and Scandinavia south

to North Africa, eastern Russia, states of the Caucasus, and northern Middle East (Alonso-Zarazaga et al. 2017). Nearctic: Canada (Alberta, British Columbia, Manitoba, New Brunswick, Newfoundland, Nova Scotia, Ontario, Prince Edward Island, Québec, Saskatchewan). United States (Alaska, California, Connecticut, Colorado, Idaho, Illinois, Indiana, Massachusetts, Minnesota, Montana, New Hampshire, New Jersey, New York, North Carolina, North Dakota, Pennsylvania, Virginia, Washington, Wisconsin). Sources: O'Brien and Wibmer (1982), Chandler (2001) and online images at bugguide.net.

Biology.—Overwintering adults become active in late May and this generation may be found into August. The next generation of adults are active from late August through September. Eggs are laid beginning in June, and larvae develop within seeds June to mid-August. Pupation typically occurs mid-August to mid-September. Egg deposition triggers a gall to form in the seeds, causing from 8 to 17 seeds to grow to around 10 times their normal size rendering them unviable (Sing et al. 2016). Mature larvae construct cells of loosely cemented fragments of the placentae which serve as pupal cases and subsequently as overwintering sites for the adults (Smith 1959).

Remarks.—First recorded in North America as an accidental introduction in 1909 (Pierce 1919), and later intentionally introduced as a biological control tool in the 1990s to control dalmatian toadflax [*Linaria dalmatica* (L.)] and yellow toadflax (*Linaria vulgaris* Mill.) (De Clerck-Floate and Harris 2002). According to molecular data, it is possible that the true *R. antirrhini* feeds only on *L. vulgaris*, whereas a cryptic new species lives on *L. dalmatica*; it is also possible that in Europe many species of *Linaria* host different taxa presently identified as *R. antirrhini sensu lato* (Hernández-Vera et al. 2010).

Rhinusa linariae (Panzer, 1792)

(Figs. 6, 12, 18)

Hosts.—Associated with various species of *Linaria* in Europe, including dalmatian toadflax (*Linaria dalmatica*) and yellow toadflax (*L. vulgaris*) (Hoffmann 1958, I. Toševski pers. comm.).

Distribution.—*Palearctic*: Europe and Russia (Alonso-Zarazaga et al. 2017). *Nearctic*: Canada (released in Alberta and British Columbia) (Bouchard 2017), United States (released in Colorado, Idaho, Montana, Oregon, and Wyoming). Apparently only limited populations have become established in Canada and the U.S. Sources: Sing et al. (2016), Wilson et al. (2005), and Winston et al. (2016).

Biology.—Overwintering adults become active in early May and this generation may be found into mid-July. The next generation of adults is active from late July through September. Eggs are laid mid-May into pockets chewed into roots and root crowns, triggering gall formation, and larvae develop within this galled root tissue from late May to early August. Pupation typically occurs late June through August. Adults often feed on stems of the host plant and before overwintering in soil or plant litter. Occasionally, fully developed adults may remain within the root galls throughout the fall and winter, emerging the following spring (Sing et al. 2016).

Remarks.—*Rhinusa linariae* was approved for release for biological control of invasive toadflaxes (*Linaria* spp.) in Canada in 1995 and 1996 (Sing et al. 2016), and after several attempts a population was established at a site in British Columbia in 1997 (De Clerck-Floate and Harris 2002). A population from British Columbia was redistributed to Colorado in 2008 (Sing et al. 2016), and

as of 2016 an established population still exists at one site in Colorado (A. Norton in litt.).

Rhinusa neta (Germar, 1821) (Figs. 4, 10, 16)

Hosts.—Associated with many species of *Linaria* in the Palearctic region, including dalmatian toadflax (*Linaria dalmatica*) and yellow toadflax (*L. vulgaris*) (Hoffmann 1958, I. Toševski pers. comm.).

Distribution.—*Palearctic*: Europe, the Caucasus, North Africa, southern Siberia, central Asia (Alonso-Zarazaga et al. 2017). *Nearctic*: Canada (British Columbia, Québec). United States (Connecticut, Indiana, Iowa, Maryland, Montana, New Jersey, New York, Oregon, Pennsylvania, Virginia, Washington). Sources: O'Brien and Wibmer (1982) and online images at bugguide.net.

Biology.—Phenology of life stages is the same as R. *antirrhini* (see above). Oviposition of eggs and larval feeding does not cause swelling of seeds, but larvae may consume a large proportion of seeds within a capsule, decreasing the seed output, but not killing the host plant (Sing et al. 2016).

Remarks.—First recorded in North America in 1937 (Buchanan 1937) as an accidental introduction, but subsequently used as a biological control agent for control of dalmatian toadflax (*Linaria dalmatica*) and yellow toadflax (*Linaria vulgaris*) (Sing et al. 2016).

Rhinusa tetra (Fabricius, 1792)

(Figs. 3, 9, 15)

Hosts.—Associated principally with common mullein (*Verbascum thapsus*), moth mullein (*V. blattaria*), and other *Verbascum* spp.; sometimes collected on *Scrophularia* spp. (Caldara 2014).

Distribution.—*Palearctic*: Europe, Siberia, North Africa, Middle East, central Asia,

and northern India. (Alonso-Zarazaga et al. 2017, Caldara 2014, Caldara et al. 2012). Nearctic: Canada (British Columbia, New Brunswick, Nova Scotia, Ontario, Prince Edward Island, Québec). United States (California, Colorado, Connecticut, District of Columbia, Georgia, Idaho, Illinois, Indiana, Iowa, Kansas, Maine, Massachusetts, Minnesota, Missouri, Montana, Michigan, Montana, Nebraska, Nevada, New Hampshire, New Jersey, New York, North Carolina, South Carolina, Ohio, Oregon, Pennsylvania, South Dakota, Texas, Utah, Vermont, Virginia, Washington, West Virginia, Wisconsin). Sources: O'Brien and Wibmer (1982), online images at bugguide.net, and MFD personal collection.

Biology.-In Washington state and Idaho, overwintering adults emerge beginning in mid-May to early June depending on elevation. Adults feed on the abaxial surfaces of leaves and on seed capsules (preferred) of the host plant. Once a plant flowers, mating occurs on the entire inflorescences. The female oviposits 1-3 eggs per seed capsule (up to 8 eggs in some capsules). Egg hatch occurs 7-11 days after deposition. Larvae feed upon developing seeds within capsules and consume the majority of seeds within. Larvae pupate within the seed capsule (generally no more than 2 per seed capsule) and emerge approximately 25 days later. New generation adults exit the seed capsule by chewing through the hardened pericarp. Thorough coverage of the biology and ecology of R. tetra is provided by Marquess (2000) from which the above account was drawn.

Remarks.—Although proposed as a potential candidate for the biological control of invasive common mullein, *Verbascum thapsus*, in North America, it is believed the species was accidentally introduced from Europe earlier than 1916 (Blatchley and Leng 1916, Majka et al. 2007).

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