

# Parasitoid complex (Hymenoptera: Eulophidae) of the leaf-mining fly *Chromatomyia horticola* (Goureau) (Diptera: Agromyzidae) in Russia

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**Abstract** Sixteen species of Eulophidae were reared from *Chromatomyia horticola* (Goureau) collected from 14 host plants in the Middle Volga Basin (Russia). *Chrysocharis viridis* (Nees), *Closterocerus trifasciatus* Westwood, *Diglyphus pusztensis* (Erdös), *Minotetrastichus frontalis* (Nees), *Neochrysocharis aratus* (Walker), *Pediobius cassidae* Erdös, and *Pnigalio pectinicornis* (Linnaeus) are new host records. Two parasitic species, *D. isaea* (Walker) and *P. metallicus* (Nees), were dominant. The pre-imaginal

stages of both dominant (ecto- and endoparasitoid) species are illustrated. The sex ratio between the ecto- and endoparasitoids differed. In June/July, there were about threefold more females in ectoparasitoids than in endoparasitoids. These differences in sex ratio were not related to the plant species only. The endoparasitoid species were found on all species of host plants of *C. horticola*, whereas the ectoparasitoid species were restricted to about half the plant species. *Diglyphus isaea* and *Pediobius metallicus* are very important regulating species against leaf miner pests such as *C. horticola*.

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## Introduction

The leaf-miner agromyzid fly *Chromatomyia horticola* (Goureau 1851) has a cosmopolitan range of distribution, damaging crops and ornamental plants in many countries throughout the world (Anon. 1987; Dempewolf 2006; Spencer 1973; von Tschirnhaus 1969). It has highly polyphagous larvae and although listed to date by Benavent-Corai *et al.* (2005) from 230 species of the dicot and monocot genera of herbaceous plants, many additional plants do not appear on the list. Spencer (1990) presented a Table of 35 host plant families. Hosts in the Brassicaceae, Fabaceae and Asteraceae are dominant for *C. horticola* (Spencer 1973, 1976, 1989, 1990; Raj *et al.* 1995). The larval

instars of *C. horticola* were described by Melis (1935) [as *Phytomyza atricornis* Meig., cf. Griffiths 1967:1-2], and further morphological characters also by Cohen (1936) and Allen (1958) (as *P. atricornis*, both could possibly also refer to the sibling species *C. syngenesiae* Hardy, cf. Griffiths 1967), as well as by M. Dempewolf (2001, Dissertation, Univ. Bielefeld, Germany). The species pupates internally at the end of the mine, with the anterior spiracles of the whitish puparium projecting through the plant epidermis (Spencer 1976). The whitish mine is linear or serpentine on both the upper and lower surface of the leaf.

In Europe and western Turkey the complex of parasitoids reared from *C. horticola* comprises 21 species of Eulophidae (Table 1). Species composition presents six species of *Chrysocharis* [*Ch. entedonoides* (Walker), *Ch. gemma* (Walker), *Ch. nephereus* (Walker), *Ch. orbicularis* (Nees), *Ch. pentheus* (Walker), *Ch. pubicornis* (Zetterstedt) (Hansson 1985; Rizzo & Massa 2002, 2004; Vidal 1997)]; six species of *Diglyphus* [*D. crassinervis* Erdős (Erdős 1958), *D. chabrias* (Walker) (Yefremova *et al.* 2011), *D. isaea* (Walker) (del Bene *et al.* 1993; Gençer 2005; Kumar 1985), *Diglyphus minoicus* (Walker), *D. pachyneurus* Graham (Gençer 2005), *D. poppoea* Walker (Rizzo & Massa 2002)]; and 2 species of *Cirrospilus* [*C. vittatus* Walker (del Bene 1989) and *C. variegatus* (Masi) (Rizzo & Massa 2002, 2004)] *Hemiptarsenus ornatus* (Nees) (del Bene 1989; Rizzo & Massa 2002), *Neochrysocharis formosus* (Westwood) (del Bene 1989; Rizzo & Massa 2002), *Omphale stigma* Goureau (Goureau 1851), *Pnigalio soemius* Walker, *P. incompletus* Bouček (Rizzo & Massa 2002), *Pediobius metallicus* (Nees) (Bouček, 1965; del Bene 1989; Civelek 2002; Gençer 2005; Rizzo & Massa 2002), *Semiela cher petiolata* Girault (Massa *et al.* 2001; Rizzo & Massa 2002). The parasitoid complexes of 15 species of Agromyzidae (including *C. horticola*) from the Middle Volga Basin were recently studied (Strakhova *et al.* 2013; Yefremova *et al.* 2012).

Taxonomically, we concur with the generic transfer of all world *Chromatomyia* Hardy, 1849 species to the genus *Phytomyza* Fallén, 1810, as recently published by Winkler *et al.* (2009). Without the provision of a detailed discussion of the male genitalia, the larval morphology, the specific mode of pupation, and the extensive published discussions

and opinions on the generic/subgeneric status, the synonymization of *Chromatomyia* (so widely used in the multilingual agricultural world literature, including handbooks) all remains highly puzzling for taxonomists and applied entomologists.

The aim of the present work was to document the parasitoid complex of *C. horticola*. This paper, which summarizes our original data and earlier published information on the eulophid species, seeks to uncover the relationships between species composition of parasitoids, *C. horticola*, and its host plants, and to analyze the ratio of ecto- and endoparasitoids of *C. horticola*, with emphasis on their development in the puparium.

## Materials and methods

The study was conducted in three adjacent locations in the Middle Volga Basin (Russia): (i) Ulyanovsk, left bank of the River Volga, Park (54°22'N, 48°32'E), (ii) Ulyanovsk Province, Dimitrovgrad (54°13'N, 49°36'E), and (iii) Ulyanovsk Province, village Lebjazhye, 90 km E of Ulyanovsk (54°06' N, 49°36'E).

*Chromatomyia horticola* mines appeared from the end of April until September in a temperate zone in Russia, with a maximum number in July, which is the warmest summer month in the study area. Leaves with *C. horticola* mines were collected from ruderal herbaceous plants near roads, in gardens, and parks from May to September 2010. This period corresponds to the seasonal peak in Agromyzidae activity, with highest growth of their host plants and highest species richness of insects in general.

Leaves were collected every 3 days from the same territory. Typical mines of *C. horticola* on leaves of white ox-eye daisy, chickory lettuce, hollyhock, and dandelion are illustrated in Figures 1–4. The collected leaves with larvae were kept in the laboratory in 0.25-liter containers under room temperature of 20–22°C. Individuals emerging from *C. horticola* mines in the containers were collected using a pooter and immersed in 75% ethanol prior to mounting and identification.

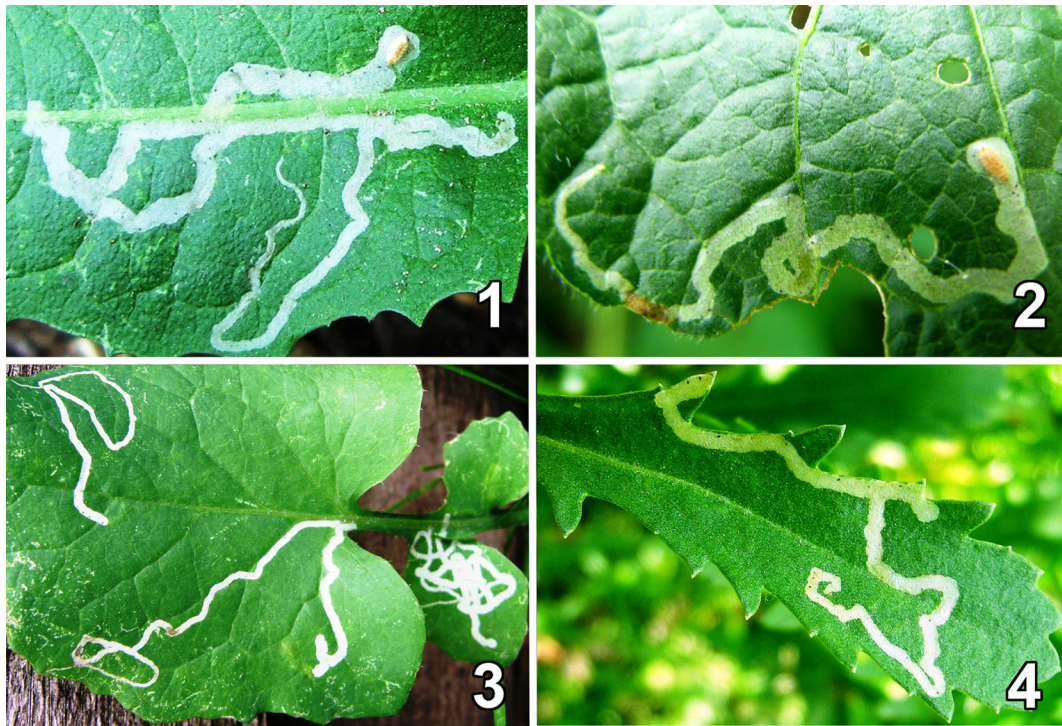
Samples were sorted using a stereomicroscope MC-2 ZOOM, and photographed using Canon Power Shot A 640. The reared Eulophidae were identified by the Russian authors. Identification keys for *Diglyphus* species are available for Europe (Yefremova & Shroll 1996), for *Chrysocharis*

**Table 1** Parasitoid species reared from *Chromatomyia horticola* mines by different authors during 1958–2011.

Parasitoid species	Ecto/endo parasitoids	Authors	Country
<i>Chrysocharis pentheus</i> (Walker)	endo	Hansson 1985	Sweden
		Ikeda 1996	Japan
		Vidal 1997	Germany
		Gençer 2009	Turkey
		Rizzo & Massa 2002	Italy
<i>Chrysocharis pubicornis</i> (Zetterstedt)	endo	Rizzo & Massa 2002	Italy
		Gençer 2009	Turkey
		Vidal 1997	Germany
		Ikeda 1996	Japan
<i>Cirrospilus vittatus</i> Walker	ecto	del Bene 1989	Italy
<i>Diglyphus crassinervis</i> Erdős	ecto	Erdős 1958	Hungary
<i>Diglyphus isaea</i> (Walker)	ecto	Kumar 1985	India
		del Bene <i>et al.</i> 1993	Italy
		Gençer 2005	Turkey
<i>Diglyphus chabrias</i> (Walker)	ecto	Yefremova <i>et al.</i> 2011	Turkey
<i>Diglyphus pachyneurus</i> Graham	ecto	Gençer 2005	Turkey
<i>Chrysocharis entedonoides</i> (Walker)	endo	Rizzo & Massa 2002	Italy
<i>Chrysocharis gemma</i> (Walker)	endo	Rizzo & Massa 2002	Italy
<i>Chrysocharis nephereus</i> (Walker)	endo	Rizzo & Massa 2002	Italy
<i>Chrysocharis orbicularis</i> (Nees)	endo	Rizzo & Massa 2002	Italy
<i>Diglyphus minoens</i> (Walker)	ecto	Rizzo & Massa 2002	Italy
<i>Diglyphus poppoea</i> Walker	ecto	Rizzo & Massa 2002	Italy
		Yefremova <i>et al.</i> 2011	Turkey
		del Bene 1989	Italy
<i>Hemiptarsenus ornatus</i> (Nees)	ecto	Rizzo & Massa 2002	Italy
		del Bene 1989	Italy
<i>Neochrysocharis formosus</i> (Westwood)	endo	Rizzo & Massa 2002	Italy
		del Bene 1989	Italy
<i>Omphale stigma</i> Goureau	endo	Goureau 1851	France
<i>Pnigalio soemius</i> Walker	ecto	Bouček & Askew 1968	Europe
		Hansson 1985	Sweden
		Rizzo & Massa 2002	Italy
<i>Pnigalio incompletus</i> Bouček	ecto	Rizzo & Massa 2002	Italy
<i>Pediobius metallicus</i> (Nees)	endo	del Bene 1989	Italy
		Bouček 1965	Europe
		Rizzo & Massa 2002	Italy
		Civelek 2002	Turkey
		Gençer 2005	Turkey
<i>Semiela cher petiolata</i> Girault	ecto	Massa <i>et al.</i> , 2001	Italy
		Rizzo & Massa 2002	Italy
<i>Zagrammosoma variegatum</i> (Masi)	ecto	Rizzo & Massa 2002	Italy

(Hansson 1985), for *Pediobius* (Bouček 1965), and for other species according to the key for the European part of Russia (Triapitsyn 1978) and the Far East of Russia (Storozheva *et al.* 1995).

A standard statistical method was used for analysis of the data. The two-way hierarchical clustering analysis with paired group was applied in order to estimate preference of parasitoids for particular plants.



**Figs. 1–4** Mines of *Chromatomyia horticola* on leaves of different host-plants: 1. Mine with puparium on *Taraxacum officinale*; 2. Mine with puparium on *Alcea rosea*; 3. Three linear mines and one serpentine mine on *Lactuca tatarica*; 4. Mine on *Leucanthemum vulgare*

Similarities between objects were measured using the Gower Similarity Coefficient.

The material is deposited in the Zoological Institute, Russian Academy of Sciences, St. Petersburg, Russia) (ZISP).

## Results

A total of 353 leaves with 379 mines were collected from 14 plant species belonging to three families: Asteraceae, Brassicaceae and Malvaceae. Most leaves housed only one mine; only 26 of the 353 leaves (6.8%) contained two mines.

The available data allow assessment of whether the species composition of the reared parasitoids is representative of the true species richness in the study area. The number of collected leaves per species of plant and the number of parasitoid species hatched from these samples is a classic example of the number of species collected and the number of samples taken (Magurran 2004). Here 90% of the parasitoid species were already

represented in the first  $102.4 \pm 12.7$  leaves, which is far less than the total of 353 leaves collected in our research.

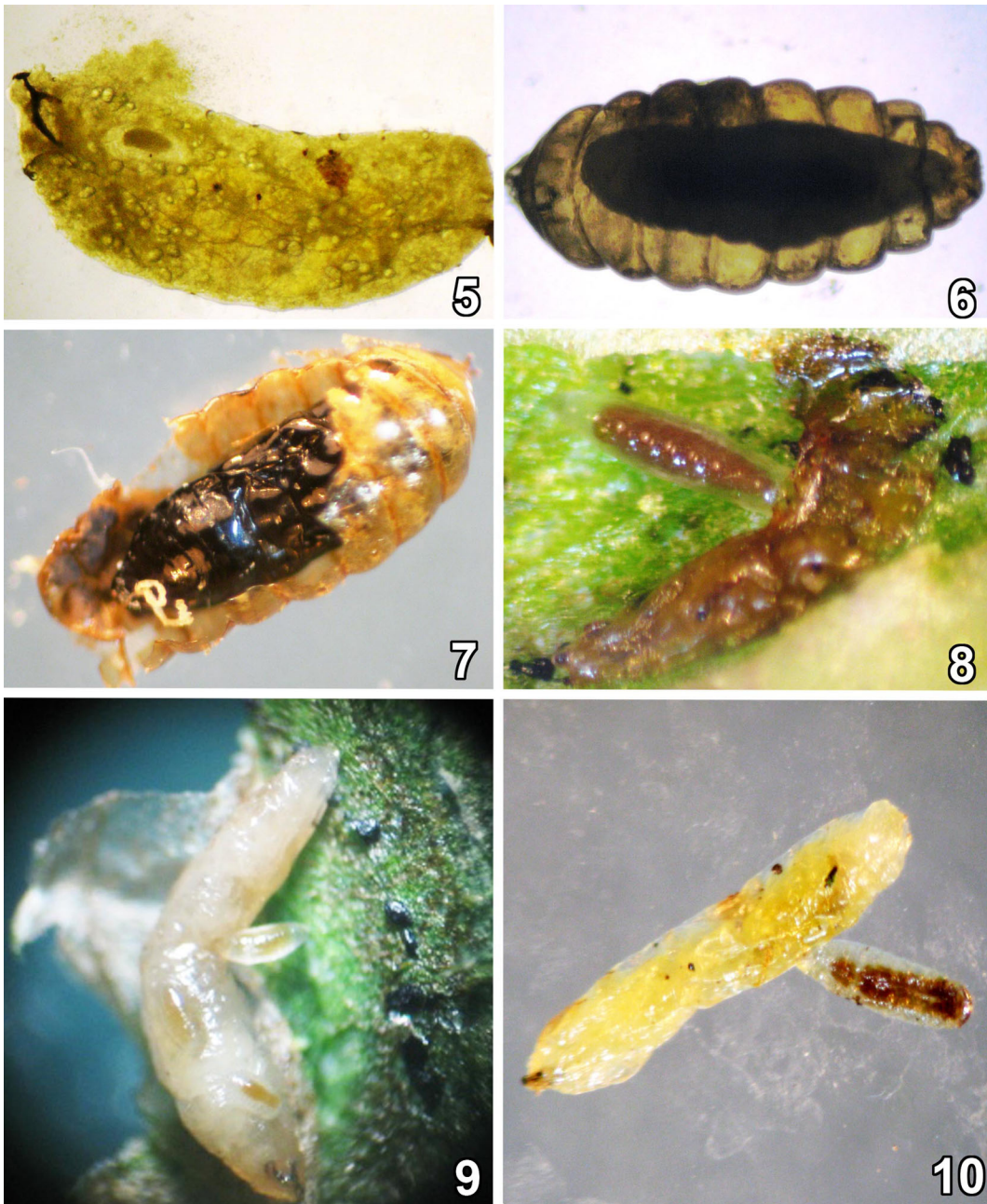
A total of 38 specimens of *C. horticola* and 349 specimens of eulophids were reared. Altogether, the revealed species assemblage comprises 16 Eulophidae species belonging to seven genera and three subfamilies (Eulophinae, Entedoninae and Tetrastichinae). The number of specimens of each species is listed in Table 2. Seven species (*Chrysocharis viridis*, *C. trifasciatus*, *D. pusztensis*, *M. frontalis*, *N. aratus*, *P. cassidae*, and *P. pectinicornis*) are new parasitoid records for *C. horticola*.

The number of specimens per species varied widely. Two dominant species, *D. isaea* (89 specimens) and *P. metallicus* (82 specimens), comprised about half (49%) of the total number of captured specimens, while fewer than ten specimens were found for many other species.

The collected parasitoids were subdivided into two ecologically different groups. Larvae of ectoparasitoids develop on the body of the host (externally), while

**Table 2** Number of parasitoid species reared from leaves with *Chromatomyia horticola* mines collected from the different host plants during 2010 in Russia.

<b>HOST PLANTS</b> n - number of leaves with mines	<i>Arctium tomentosum</i> Mill. N = 30	<i>Artemisia absinthium vulgare</i> L. n = 10	<i>Artemisia vulgaris</i> L. n = 55	<i>Callistephus chinesis</i> (L.) n = 5	<i>Carduus crispus</i> L. n = 10	<i>Centaurea sumensis</i> Kalen. N = 25	<i>Cirsium arvense</i> (L.) n = 30	<i>Cirsium vulgare</i> (Savi) n = 12	<i>Lactuca tatarica</i> (L.) n = 16	<i>Leucanthemum vulgare</i> Lam. N = 45	<i>Sonchus arvensis</i> L. n = 5	<i>Taraxacum officinale</i> Web. ex Wigg n = 5	<i>Sisymbrium Loeselii</i> L. n = 90	<i>Alcea rosea</i> L. n = 15	<b>Total:</b>
<b>PARASITOID SPECIES</b>															
<b>Eulophinae</b>															
<i>Diglyphus chabrias</i> (Walker)								1 ♀							1
<i>D. crassinervis</i> Erdős	3 ♂		2 ♀	1 ♀					3 ♀	1 ♀					10
<i>D. isaea</i> Walker	18 ♀, 1 ♂	3 ♀, 1 ♂	6 ♀, 3 ♂				1 ♀		3 ♀		1 ♀, 1 ♂		40 ♀, 11 ♂		89
<i>D. poppoea</i> Walker							2 ♂		1 ♀	23 ♀, 4 ♂					28
<i>D. pusztensis</i> (Erdős, Novicky)	9 ♀														11
<i>Pnigallo pectinicornis</i> (Linnaeus)	1 ♀														1
<i>P. soemius</i> (Walker)	13 ♀		28 ♀, 6 ♂		1 ♀	7 ♀, 2 ♂	2 ♀	1 ♂							60
<b>Entedoninae</b>															
<i>Chrysocharis crassiscapus</i> (Thomson)							1 ♀								1
<i>Ch. pubicornis</i> (Zetterstedt)		1 ♀					1 ♀								2
<i>Ch. viridis</i> (Nees)							1 ♀, 17 ♀, 1 ♂								1
<i>Closterocerus trifasciatus</i> Westwood													3 ♀		3
<i>Neochrysocharis aratus</i> (Walker)									1 ♀				2 ♀, 1 ♂	2 ♀	15
<i>N. formosus</i> (Westwood)		2 ♀, 1 ♂	4 ♀		1 ♀	2 ♀	3 ♀	1 ♀					7 ♀, 1 ♂	5 ♀, 1 ♂	22
<i>Pediobius cassidae</i> Erdős					1 ♂	2 ♀, 5 ♂	3 ♀, 3 ♂	2 ♀, 1 ♂	1 ♂	9 ♀, 2 ♂			16 ♀, 28 ♂	2 ♀, 1 ♂	82
<i>P. metallicus</i> (Nees)	1 ♂		2 ♀, 3 ♂												
<b>Tetrastichinae</b>															
<i>Minotetrastichus frontalis</i> (Nees)			1 ♂											2 ♀, 1 ♂	5
Total parasitoids individuals	46	7	55	1	4	20	29	10	11	46	2	3	101	11	349
Total parasitoid species	6	2	6	1	4	4	5	6	6	3	1	3	6	3	
Total <i>Chromatomyia horticola</i> individuals	1 ♂	1 ♀	1 ♀, 2 ♂	1 ♂	2 ♀, 1 ♂	1 ♂	1 ♂	1 ♂	6 ♀, 2 ♂	8 ♀, 3 ♂	1 ♂	1 ♀	2 ♀, 1 ♂	1 ♀, 1 ♂	38



**Figs. 5–10** Development of parasitoids on/in larva/pupa of *Chromatomyia horticola*: 5. Larva of *C. horticola* with first instar endoparasitoid larva of *Chrysocharis* sp. on *Leucanthemum vulgare*, 6. Pupa of *C. horticola* with last instar larva of *Pediobius metallicus* on *Artemisia vulgare*, 7. Pupa of *C. horticola* with endoparasitoid pupa of *Pediobius metallicus*

on *Artemisia vulgare*, 8. Ectoparasitoid larvae of *Diglyphus* sp. on larva of *C. horticola* and endoparasitoid inside body on *Artemisia vulgare*, 9. Three *Diglyphus crassinervis* larvae of different ages on larva of *C. horticola* on *Callistephus chinensis*, 10. Ectoparasitoid larva of *Diglyphus* sp. on larva of *C. horticola* removed from mine of *Leucanthemum vulgare*

larvae of endoparasitoids develop inside the host. Some of the pre-imaginal stages of both dominant (ecto- and endoparasitoid) species are shown in Figures 5–10. A fully developed endoparasitoid is almost equal in size to

that of the host and occupies almost the entire volume of the body of its host except for the head capsule. In contrast, ectoparasitoids are always smaller than their host. Although several conspecific ectoparasitoids at

different developmental stages might feed together on the body of a dipterous larva without competitive behavior, the competitive presence of two parasitoid species on or in the body of a host larva has also occasionally been observed.

Ectoparasitoids were represented in our species assemblage by genera belonging to the Eulophinae and Tetrastichinae, with eight species and 205 specimens (58.7% of total). Endoparasitoids were represented by genera of the Entedoninae, with eight species and 144 specimens (41.3%).

The majority of the parasitoid specimens (88.5%) emerged in June and July. The number of ecto- and endoparasitoids in this period was similar, around 50%  $\pm$  12% each. However, the sex ratio within these groups differed: in ectoparasitoids there were 4.2-fold and 4.4-fold more females than males in June and July, respectively, whereas for endoparasitoids these values were only 1.2-fold and 1.5-fold.

For example, the dominant species, *D. isaea*, was characterized by 3–5-fold more females than males in summer: in June the ratio was 38 ♀/11 ♂, and in July 32♀/6♂. In the dominant endoparasitoid, *P. metallicus*, males outnumbered females by 25 ♂/18 ♀ in June and by 16♂/14♀ in July. In other words, 1.3-fold and 1.1-fold more males emerged than females (Fig. 11). These differences in sex ratio between the ecto- and endoparasitoids were probably not related to the plant species. On the small tumble-mustard (*Sisymbrium loeselii*), a plant of the cabbage family, the ratio for *D. isaea* in June was 26 ♀/6 ♂ and in July 14♀/5♂, whereas for *P. metallicus* in June it was 6 ♀/15 ♂ and in July 10♀/13♂.

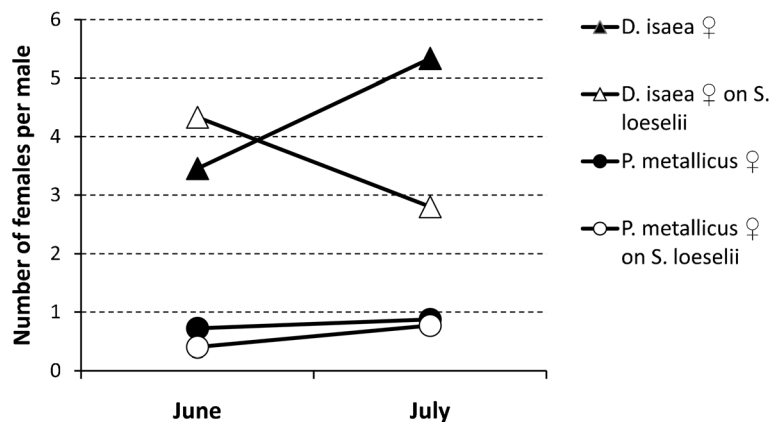
To determine whether ecto- and endoparasitoids preferred a particular species of plant, eight parasitoid

species with more than ten emerged specimens were chosen. According to their preference for a particular host plant, the parasitoids were divided into two main branches, separated at the 0.6 level of Gower distance (Fig. 12). Species composition between these branches separated into the ecto- and endoparasitoids. One branch contains the endoparasitoids (*N. formosus*, *C. trifasciatus*, *P. cassidae*, *P. metallicus*), and the other the ectoparasitoids (*P. soemius*, *D. isaea*, *D. poppoea*, *D. pusztensis*). These two branches cover different ranges of plants. Endoparasitoids were found on all collected species of plants, while ectoparasitoids were apparently limited to only about half of them: namely, *A. tomentosum*, *A. vulgaris*, *C. vulgare*, *L. tatarica*, and *C. sumensi*.

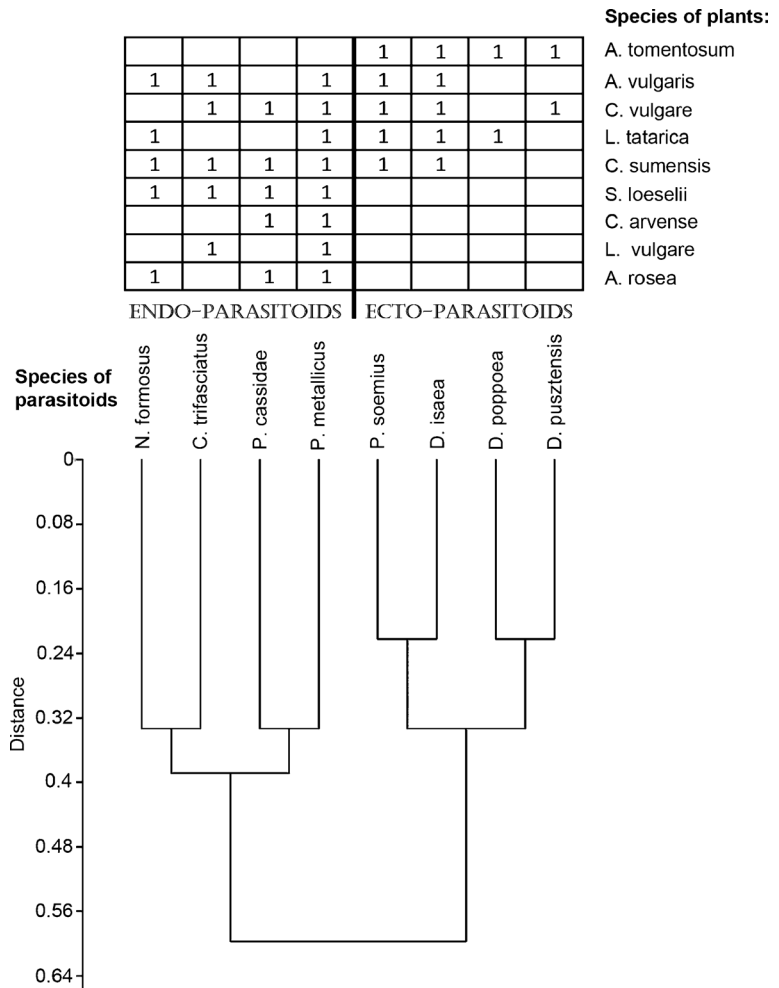
## Discussion

More than 21 species of eulophids (12 ecto- and nine endoparasitoids) are known as parasitoids of *C. horticola* in Europe (see Introduction). Of these, only 11 species were included in this study. In contrast to previous studies, we omitted species of the genera *Cirrospilus* and *Semielaecher* because species of *Cirrospilus* have never been reared from *C. horticola* in the Middle Volga Region and species of *Semielaecher* are not known at all from Russia (Yefremova 2002). *Omphale stigma*, which was reared in France 162 years ago (Goureau 1851) but never again, was also omitted from the list. The species *Pnigalio soemius* is considered a valid parasitoid of *C. horticola* and was reared from *C. horticola* in our study as well as in other studies (e.g. Bouček & Askew 1968). In contrast to these results, Gebiola *et al.* (2012) reared this species from

**Fig. 11** Sex ratio in the ectoparasitoid *Diglyphus isaea* and the endoparasitoid *Pediobius metallicus* throughout the 2010 summer season in Russia



**Fig. 12** Two-way clustering preference of parasitoids for particular plants. Similarity measured by Gower Similarity Coefficient; algorithm is paired group. Data transformed to presence (1), absence (0 = empty cell).



lepidopterous hosts and considered it a cryptic species. *Neochrysocharis formosus* is also accepted as a valid species, as confirmed by molecular investigation by Tetsuya *et al.* (2011).

The dominant species of the parasitoid complex of *Chromatomyia fuscata* Zetterstedt was *Diglyphus begini* (Ashmead) (Hågvar *et al.* 2000) and of *Agromyza frontella* (Rondani) – *Diglyphus* species (Coote & Ellis 1986); consequently it is an ectoparasitoid species. Heinz & Parrella (1990) showed for *D. begini* that females oviposit "female-eggs" on a large host of *Liriomyza trifoli* (Burgess), while "male-eggs" are oviposited on smaller hosts. The size of the host larvae was not measured in our study, but the number of females was usually 3–5-fold greater than that of males.

*Pediobius metallicus* was not a common species in the parasitoid complex of *C. fuscata* (Hågvar *et al.* 1998) and it is mentioned in our study for the first time

as a dominant species of *Chromatomyia* spp. in general. The sex ratio in the genus *Pediobius* observed for *P. foveolatus* reared from Coccinellidae, was revealed as 1♂:1.33♀ for field-collected parasitoids and 1♂:6.75 for laboratory-reared ones, with an average ratio of 1♂:3.29♀ (Stevens *et al.* 1977). In our study the sex ratio of *P. metallicus* was 1.3♂:1♀. The sex ratio of *P. metallicus* reared from *C. horticola* is presented for the first time.

The terms idiobionts and koinobionts as understood by Askew & Shaw (1986), are not included in this paper because these species of Eulophidae (in our list of the parasitoid complex of *C. horticola*) coincides with the concepts ecto- and endoparasitoids. The differences in the association of the ecto- and endoparasitoids with particular species of plants probably derive from the sensitivity of parasitoid larvae to environmental factors. Endoparasitoids develop inside the bodies of their host



larvae and are therefore protected by the homeostasis of the larvae from the influence of environmental conditions; whereas ectoparasitoid larvae develop outside the host's body, thus being more exposed to the influence of environmental factors.

The dominant species *Diglyphus isaea*, like *D. begini* (Hågvar *et al.* 2000), is a very important regulating species and is widely used as an agent against agromyzid leaf miner pests in biological control programs. The species *P. metallicus* may also be considered in future as an agent against leaf miner pests.

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