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Actual and potential role of parasitoids (Hymenoptera: Eulophidae) in control of water-lily beetle *Galerucella nymphaeae* (Coleoptera: Chrysomelidae) in conditions of Botanical Garden of P.J. Šafárik University in Košice (Slovakia)

MARTIN SUVÁK [1]*, ROBERT GREGOREK [1] & MARTINA PĽUCHTOVÁ [2]

[1] Botanical Garden of Pavol Jozef Šafárik University in Košice, Mánesova 23, 043 52 Košice, Slovakia; martin.suvak@upjs.sk, greko@gmail.com

[2] Technical University of Košice, Letná 9, 042 00, Košice

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Abstract: In the Botanical Garden of P.J. Šafárik University in Košice the water-lily beetle *Gallerucella nymphaeae* has been causing serious problems in growing ornamental cultivars of water-lilies *Nymphaea* x *hybrida*. When looking for possibilities of natural regulation of the beetle, the occurrence of egg parasitoids has been found with predominance of *Oomyzus gallerucae*. Later on, the occurrence of *Aprostocetus celtidis* has been noticed occasionally and the structure of hatching results suggests the possibility of its hyperparasitic relationship with *O. galleru*cae. This is the first record of association of these chalcid wasps to *G. nymphaeae* as well as the first ever registered species of parasitoids of the water-lily beetle eggs. During summer of 2012, the parasitation rate had been watched in three consequent extensive sampling events. The rate of parasitized eggs from individual basins varied from 2 % to 29 %, 12% being average.

To compare, and potentially to enrich the spectrum of parasitoids in biological control of water-lily beetles in BG PJŠU, the nearest known natural habitats with occurrence of *G. nymphaeae* were

*corresponding author

visited near Klin nad Bodrogom. The samples of water-lily beetles provided pupal parasitoids – species *Asecodes lucens* and *Tetrastichus clito*, which have not been found from Košice samples yet. In the case of *T. clito*, it is another first ever record of parasitic relationship of this species with the water-lily beetle *G. nymphaeae*.

From the faunistic point of view, all four species of chalcid wasps were recorded in Slovakia for the first time.

Three newly registered associates (*O. gallerucae, A. celtidis* and *T. clito*) of *G. nymphaeae* will need further evaluation. According to literary sources, the typical regulator of the water-lily beetle seems to be *A. lucens*. Its introduction to BG PJŠU could significantly help in the biological protection of water-lilies grown there. The possibility of wider application of all parasitoids should be studied with regard to occurrence of alternative hosts on vegetation around the basins. As the registered eulophid species behave as generalist parasitoids, the differences in their chrysomelid hosts (and other parasitoids in the case of *A. celtidis*) and associated food plants should be taken into account in next research there.

Keywords: *Nymphaea* x *hybrida*, *Galerucella nymphaeae*, Eulophidae, *Oomyzus gallerucae*, *Aprostocetus celtidis*, *Asecodes lucens*, *Tetrastichus clito*, biological control, botanical garden

Introduction

Insect herbivory can considerably affect aquatic plants with floating leaves (STENBERG & STENBERG 2012). Leaf beetles (Chrysomelidae) of genus *Galerucella* Crotch, 1873 are being used or are planned to be used in some countries as a biological control tool in regulation of some invasive plants, often including some unwanted water and marsh plant species (macrophytes) (e.g. DING & BLOSSEY 2005). Water-lily growers, however, have to face the opposite problem since the leaf beetle *Galerucella nymphaeae* (Linnaeus, 1758) is able to degrade various cultivars of Nymphaeaceae taxa in garden water elements (Figs. 1, 2, 3).

Representatives of Nymphaeaceae family, including numerous decorative varieties of water-lilies, have been grown in the Botanical Garden of P. J. Šafárik University in Košice (thereinafter BG PJŠU) for a long time. (GREGOREK 2011). So far a wide variety of biotic harmful factors has been observed on them. Insect pests are mainly represented by aphids *Rhopalosiphum nymphaeae* (Linnaeus, 1761), visible feed marks on the leaves were caused mainly by larvae of nonbiting midges *Cricotopus* sp. van der Wulp, 1874 and leaf beetles *Donacia crassipes* Fabricius, 1775. In the last five years the most significant pest seemed to be leaf beetle *G. nymphaeae*, probably introduced into the BG PJŠU with new cultivars of water-lilies from external source (Prague, Czech Republic). Since the first observation of its extensive occurrence (2008) this leaf beetle has yearly caused massive damage to leaf area of the water-lilies grown (Fig. 3). Standard

Fig. 1. Basin no. 1 with water lilies (*Nymphaea* sp., in the water) and potted marsh plants (on the pyramid) in BG PJŠU.

Fig. 2. Basin no. 2 (in the front) with water lilies (*Nymphaea* sp.) and closely neighbouring Basin no. 3 (at the back) with yellow water lilies (*Nuphar lutea*) and other water and marsh plants in BG PJŠU.

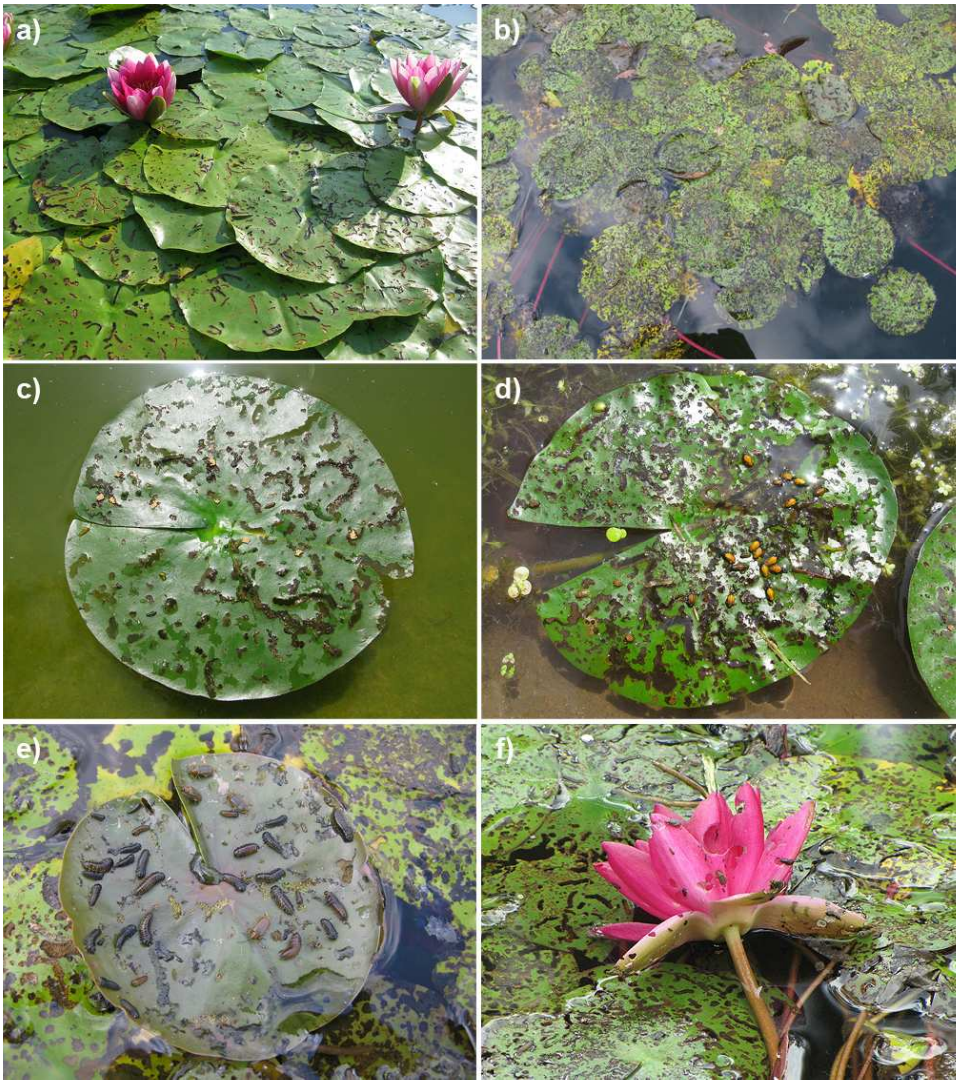


Fig. 3. Damage to water lily leaves and the occurrence of various stages of *Galerucella nymphaeae*: a) first marks of damage on *Nymphaea* hybr. 'Charles de Meurville', b) badly damaged leaves of *N.* hybr. 'Perry´s Beauty', c) water lily leaf with 9 egg clutches , d) a leaf (*N. alba*) with numerous fresh pupae, e) grown up larvae and adults coming to a young leaf from the surrounding badly damaged and flooded leaves, f) after badly damaging the leaves, the larvae and adults had also attacked the water lily flower (*N.* hybr. 'James Brydon').

pesticides cannot be applied there, among others due to fish presence in water. The other measures used there proved to be ineffective (experimental application of *Bacillus thuringiensis tenebrionis* based agent), or extremely time consuming (manual disposal of various stages of leaf beetles on the leaves). This lead to investigating other options of natural regulation based on knowledge of natural enemies of this leaf beetle in BG PJŠU as well as in surrounding natural habitats.

When comparing various works on ecology of leaf beetle of *Galerucella* genus, several problems of the host identity need to be taken into account. For example, according to NOKKALA et al. (1998), the American and European populations of *G. nymphaeae* are separate species. Some authors consider *G. nymphaeae* (American population) to be polyphagous species where the food preferences of individual subpopulations are relatively stable (CRONIN et al 1999). The other authors deal with the so called *Galerucella nymphaeae* complex (European populations), into which some specific types belong. They are narrowly defined on the basis of little morphological differences or eventually with respect to their occurrence on different host plants. BORGHUIS et al. (2009) compared different samples of *Galerucella* beetles on the basis of their differences in the chosen DNA fragments and considering their occurrences on various host plant species. The representatives of *Galerucella nymphaeae* complex, according to them, are closely related lines on different groups of host plants (Lythraceae, Polygonaceae, Nymphaeaceae and Rosaceae). According to WARCHAŁOWSKI (2003), the European complex of *Galerucella nymphaeae* includes *G. nymphaeae* (s.str.), *G. aquatica* (Geoffroy in Fourcroy, 1785), *G. sagittariae* (Gyllenhal, 1813) and *G. kerstensi* Lohse, 1989. NESTEROVA (2008) states that very similar species *G. nymphaeae* and *G. aquatica* can appear in the same locality but, next to the specific morphological differences of adults and larvae, they also differ by their occurrence on host plants – *G. nymphaeae* on the plants of genera *Nymphaea* and *Nuphar* (Nymphaeaceae) while *G. aquatica* occurs on *Rumex* sp. (Polygonaceae). Unless otherwise specified, in the following text we consider *G. nymphaeae* (Fig. 4) just in such sense, like narrowly defined European species associated with Nymphaeaceae plants.

The literary sources contain data on various kinds of natural enemies of *G. nymphaeae*, mainly predatory and parasitic invertebrate species. Generally, various stages of *G. nymphaeae* can be attacked by wide variety of unspecified predators but for many of them the prey on the floating leaves of water-lilies or yellow water-lilies is hard to access. In Northern America some predatory bugs (Hemiptera: Gerridae, Notonectidae) and ladybirds (Coleoptera: Coccinellidae) were evaluated from this viewpoint (DING & BLOSSEY 2005). SCHLACHER & CRONIN (2007) also discussed the significant influence of selected ladybird predators in reducing grazing damage caused by *G. nymphaeae* to *Nuphar advena* leaves in USA. NOYES (2012) presents only the following chalcidoid parasitoids of this leaf beetle species complex: *Asecodes lucens* (Nees, 1834) and unspecified type of *Pediobius* sp. Walker, 1846 (Hymenoptera: Eulophidae: Entedontinae) - they both are parasitoids of larvae, resp. pupae, no egg parasitoids are mentioned there. However, it can be supposed that some oligophagous parasitoids attacking the related species of *Galerucella* genus (or eventually representatives of related genera) could possibly develop themselves on *G. nymphaeae,* too*.* The presented paper deals with this idea and provides the first results on parasitoids from Eulophidae family developing in stages of *G. nymphaeae* beetles feeding on the water-lilies and yellow water-lilies in BG PJŠU and its surroundings.

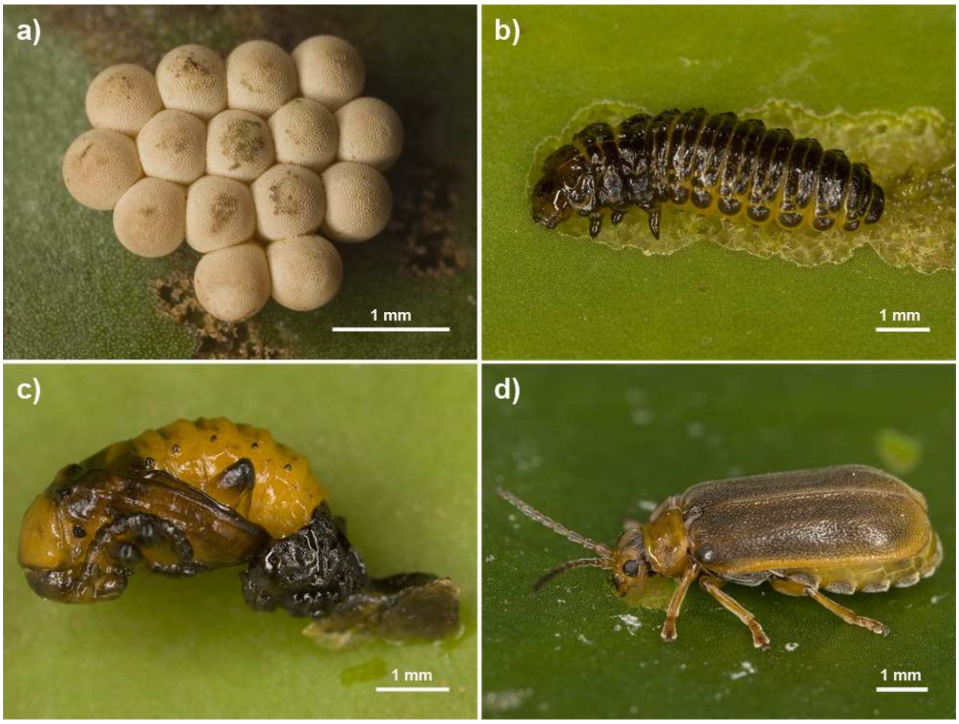


Fig. 4. Details of stages of *Galerucella nymphaeae* on water lily leaves: a) eggs, b) older larva, c) pupa, d) imago.

Material and methods

Characteristics of monitored sites

BG PJŠU is situated on the outskirts of Košice, the second largest city in Slovakia. Its coordinates are 48°44' 08'' N, 21°14' 17'' E, the altitude ranges from 210 m to 370 m above sea level. South-eastern headland of the Volovské vrchy Mts. extends to the Košice Basin in the place of BG PJŠU location. In the lower, plane area of the garden, there are three basins where water plants are grown. At the time of vegetation season 2012, *Nuphar lutea*, *Nymphaea alba* and following cultivars *Nymphaea* x *hybrida*: 'Charles de Meurville', 'Rosennymphe', Průhonice hybrid, 'Perry´s Beauty', 'Gold Medal', 'Sioux', 'James Brydon', 'Burgundy Princess', 'Gladstoniana', 'Gloire du Temple sur Lot' and 'Marliacea Chromatella' were grown there. Basin no. 1 (Fig.1) is situated separately on the flat surface. In the middle of the basin there is an elevated stage pyramid of the fountain and along the basin circumference, in the vegetation area, the pots with various water-lilies cultivars (mentioned above) are placed under water on the bottom of the basin. The water is discharged from this basin in winter time and pots with water-lilies are removed indoors. Around the basin there are regularly mown lawns with scattered plantings of bushes and smaller trees. Basins no.2 and no.3 (Fig. 2) are situated close to each other, both being 90 metres distant from Basin no.1. They are under the slight grass slope, there are bushes and tall trees nearby. Basin no. 2 is just below the Basin no.3, during the season mainly water-lily cultivars are grown there (*Nymphaea* hybr. – mentioned above). Basin no. 3 contains a wide variety of water and marsh plants of various families all the year, but out of Nymphaeaceae only plants of yellow water-lily (*N. lutea*) are present there.

The nearest known natural habitat with actual occurrence of white water-lily (*N. alba*), yellow water-lily (*N. lutea*) and leaf beetle *G. nymphaeae* is the dead branch of the river Bodrog (Fig. 5) near the border with Hungary in the territory of Klin nad Bodrogom village: 48°22' 08'' N, 21°42' 18'' E, the altitude of 94 m above sea level. Bee-line distance of both habitats (Košice – Klin nad Bodrogom) is approximately 53 kilometres.

Fig. 5. Oxbow lake of the Bodrog river near Klin nad Bodrogom with natural occurrence of *Galerucella nymphaeae* beetles on *Nymphaea alba* and *Nuphar lutea.*

Collecting and processing of material

In the summer season of 2012, the adults as well as larvae of leaf beetle *G. nymphaeae* were mechanically disposed along with the most damaged water-lily leaves. It was a common phytosanitary procedure carried out to reduce the damage to the cultivars grown in the basins. A part of collected stages of beetles with leaves was used to study the occurrence of parasitoids in comparison with the previous years when all such material was disposed by responsible workers of BG PJŠU. During the three sampling events (2nd July 2012, 7th August 2012 and 23rd August 2012) the individual clutches of eggs laid on the leaves were cut out with the leaf tissues and placed into the numbered vials. Some samples of adults and larvae were also collected in smaller extent. The vials with the leaves and eggs were kept in the room temperature of approximately 25 °C for about two weeks and checked regularly after each 1 to 3 days. When necessary, the samples were humidified or the mold coatings were removed from them. The hatched larvae of water-lily beetles and adults of parasitoids were counted. The part of hatched chalcid wasps was killed in the ethyl acetate vapours and the other part was placed into the alcohol. All the samples were marked with details of their origin and dates of collecting egg clutches and hatching of individuals.

At the end of vegetation season, on 18th September 2012, the first preliminary visit of the nearest natural habitat with massive occurrence of *N. lutea* and *N. alba* near Klin nad Bodrogom was organized. Samples of all stages of *G. nymphaeae* were collected to determine the possible presence of parasitoids. These samples were further processed in the same way as samples collected in BG PJŠU.

The adults of the hatched chalcid wasps were determined according to GRAHAM (1985, 1991) and TRJAPITZIN (1978). The names used in the article are according to NOYES (2012).

A part of samples was prepared to be scanned with scanning electron microscope FE SEM MIRA3 TESCAN at the Institute of Geotechnics of SAS in Košice.

Results

Prior to systematic collecting water-lily beetle samples in BG PJŠU in Košice as well as during the first sampling in natural habitat near Klin nad Bodrogom, the species identity of phytophagous leaf beetle (Fig. 4) was checked on the observed plants of Nymphaeaceae family. In both localities, based on the morphological character of adults, all collected leaf beetles were confirmed to be *G. nymphaeae* according to WARCHAŁOWSKI (2003).

From both basins (no's. 1 and 2 in Figs. 1, 2) in BG PJŠU 3446 eggs in total were collected from 269 egg clutches, the average number of eggs per laying (egg clutch) was 12.8. In the time of collecting, some eggs had already been hatched. Only the full eggs were used for further comparison whose number was 1979 in total. During the checking of eggs, hatching the chalcid wasps occurred in various extent besides the beetle larvae (Fig. 6). Initially it was exclusively *Oomyzus gallerucae* (Fonscolombe, 1832) (Hymenoptera: Eulophidae: Tetrastichinae) (Fig. 7), but in the third series of samples from Basin no. 1 a few

Fig. 6. Individuals hatching from collected egg clutches of *Galerucella nymphaeae*: a) larvae of *G. nymphaeae*, b) imagoes of *Oomyzus gallerucae*.

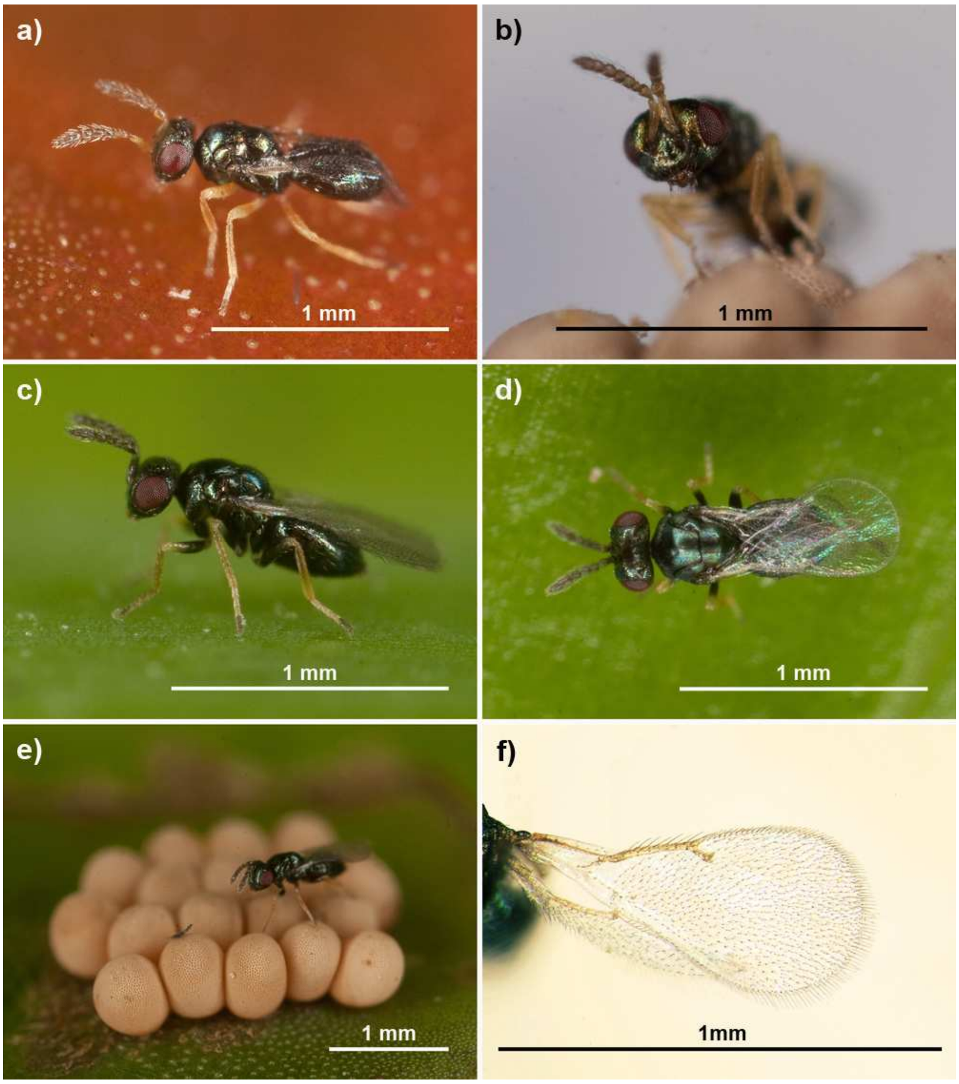
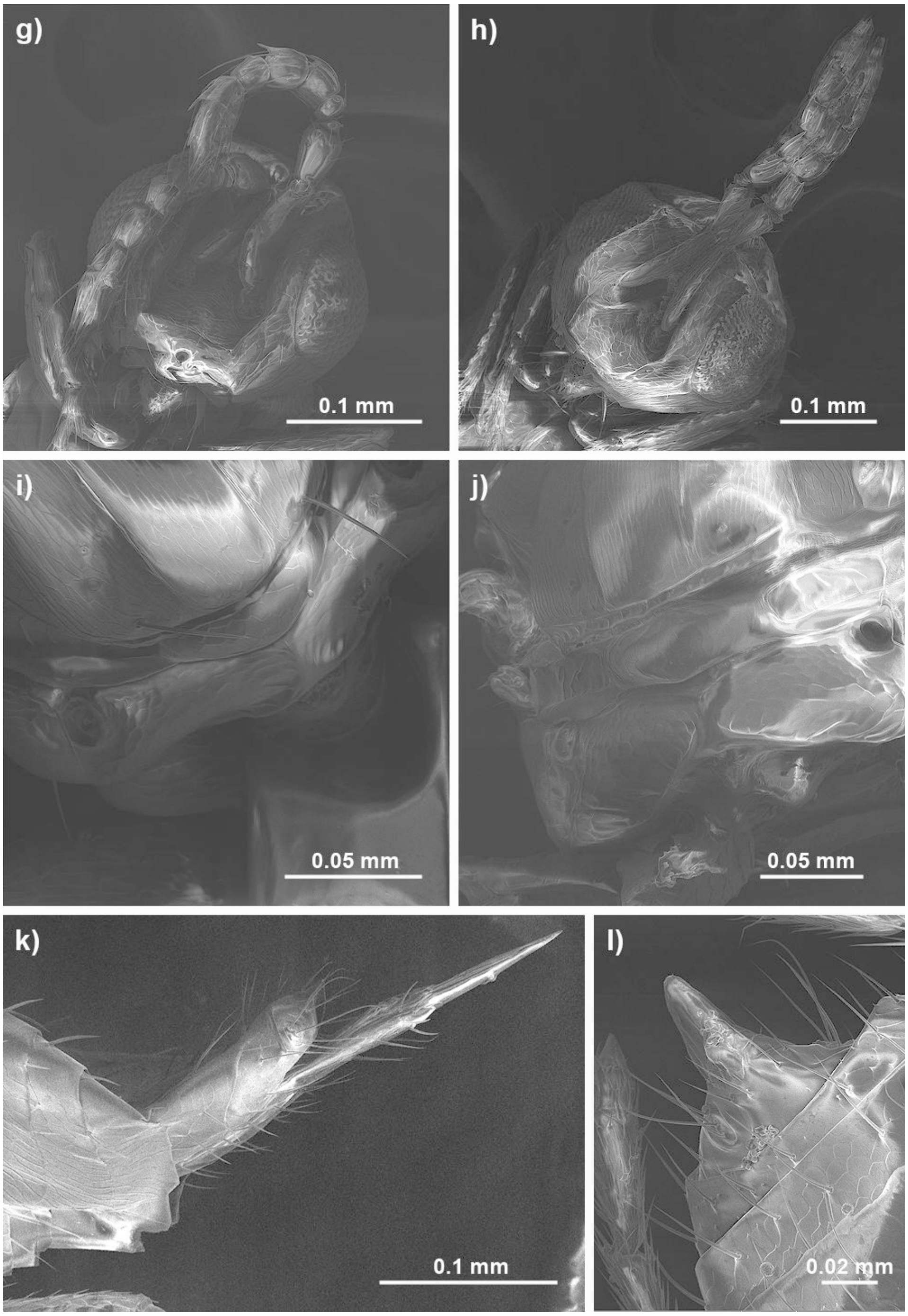


Fig. 7. ***Oomyzus gallerucae.***

males: a), b), g);
females: c) – f), h) – l);

a), c) – body in lateral view; b), g), h) – frontal views of heads; d) – body in dorsal view; e) – female on egg clutch of *Galerucella nymphaeae*; f) – right fore wing from above; i) – caudal part of scutellum, dorsellum and propodeum from above; j) – caudal part of scutellum, dorsellum and propodeum in caudal view; k) – ovipositor, lateral view; l) – tip of abdomen and cercal setae, dorsal view.


g)
0.1 mm
h)
0.1 mm
i)
0.05 mm
j)
0.05 mm
k)
0.1 mm
l)
0.02 mm

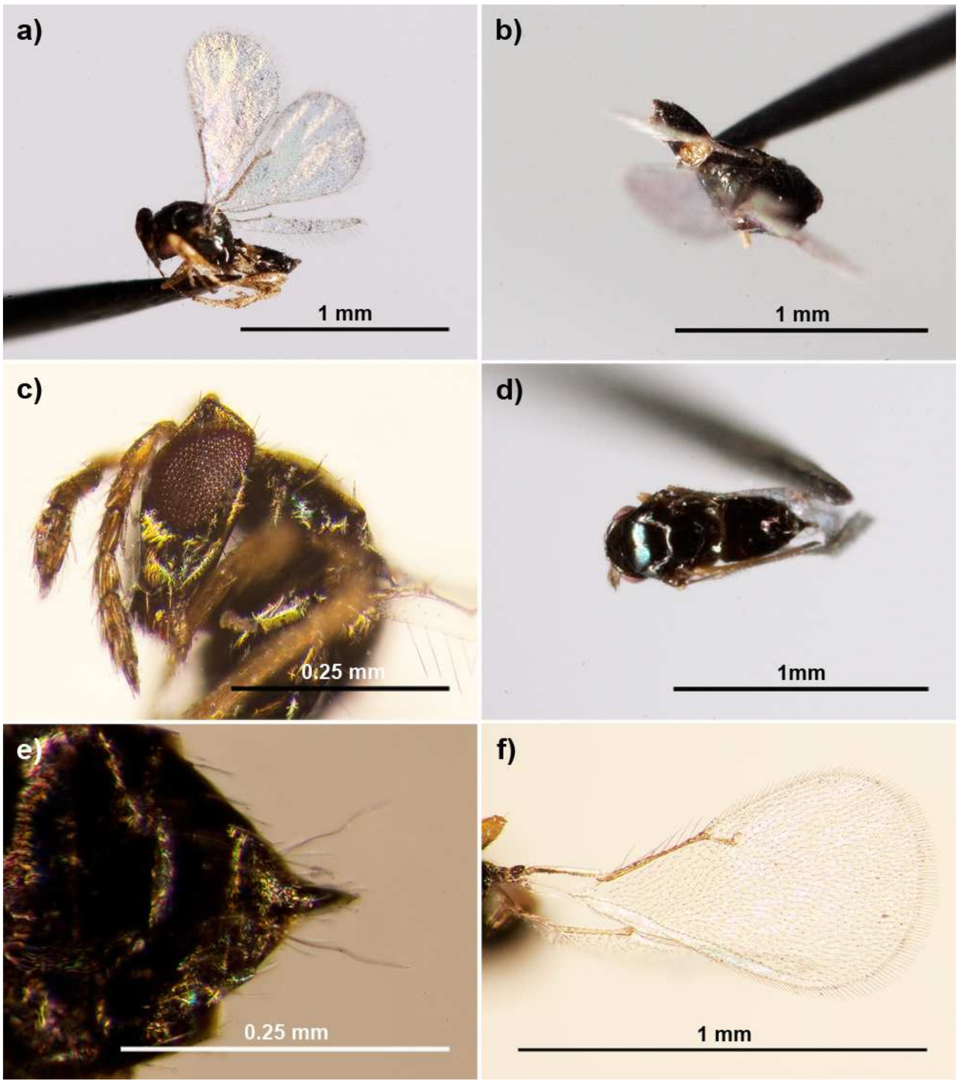
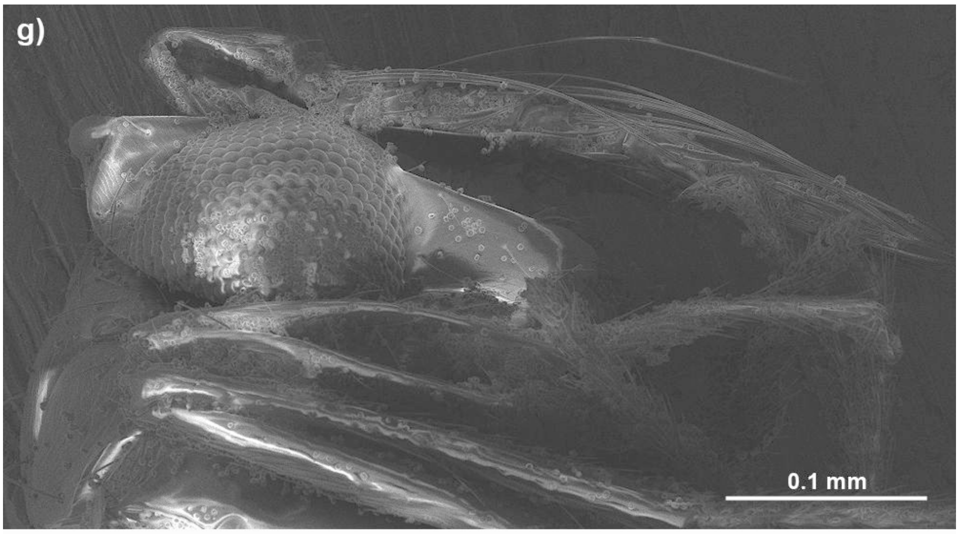
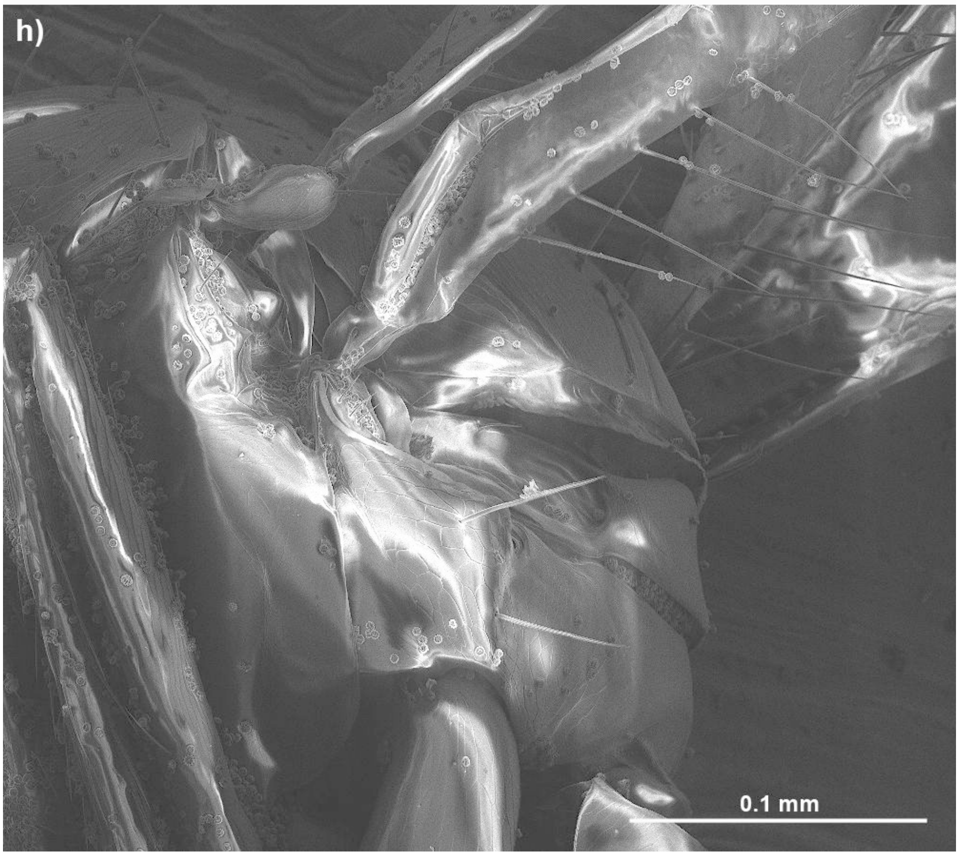


Fig. 8. ***Aprostocetus celtidis*.**

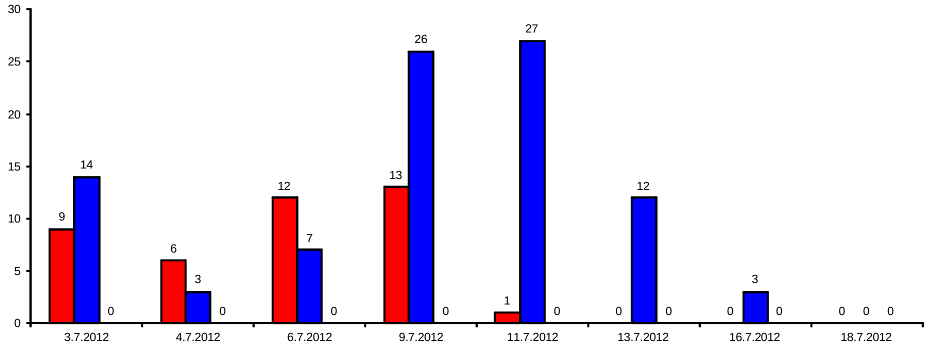
males: a), b), g);
females: c) – f), h);

a) – body in lateral view; b), d) – body in dorsal views; c), g) – heads in lateral views; e) – abdominal tip and cercal setae from above; f) – right fore wing from above; h) – scutellum, dorsellum and propodeum, caudo-lateral view.

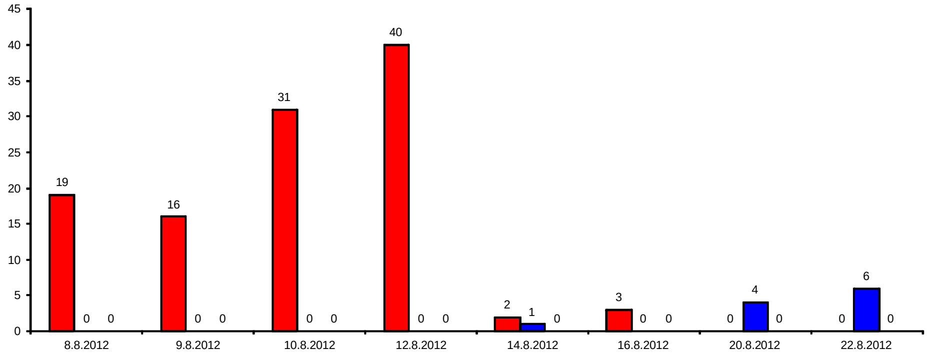

g)
0.1 mm


h)
0.1 mm

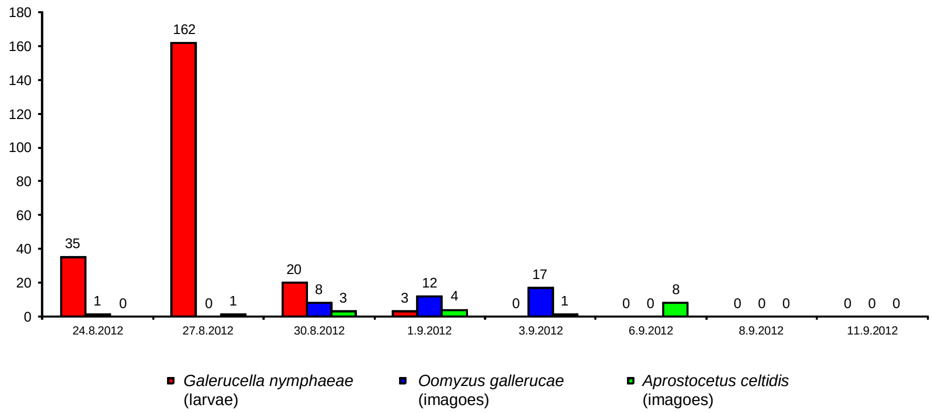
a) Basin no.1:
Distribution of **133** different individuals gradually hatched from **313** full eggs of *Galerucella nymphaeae* collected on **2.7.2012**



b) Basin no.1:
Distribution of **122** different individuals gradually hatched from **186** full eggs of *Galerucella nymphaeae* collected on **7.8.2012**

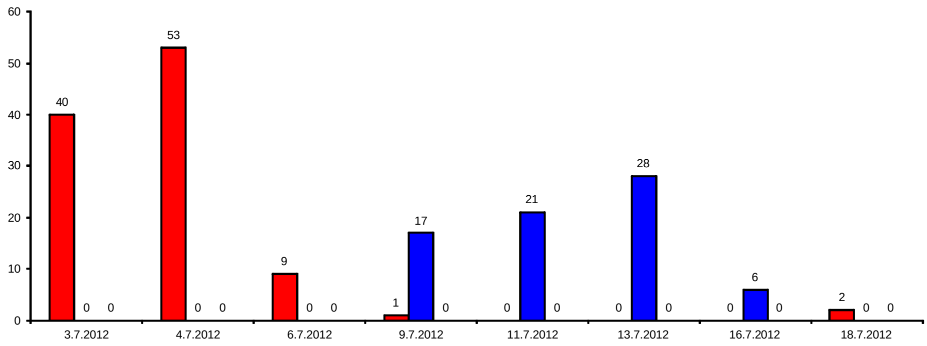


c) Basin no.1:
Distribution of **276** different individuals gradually hatched from **411** full eggs of *Galerucella nymphaeae* collected on **23.8.2012**



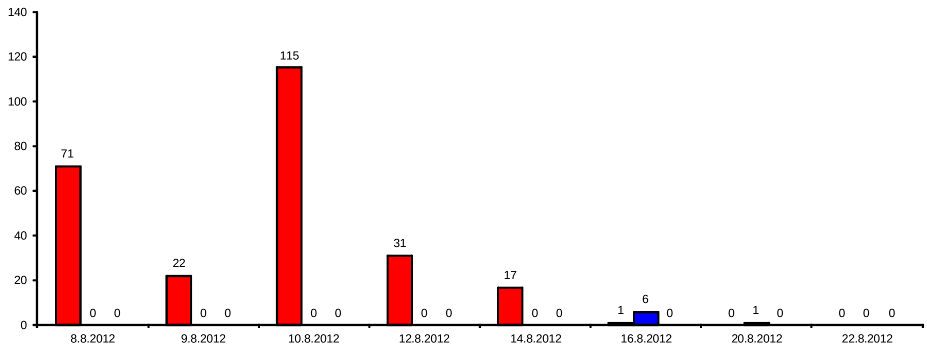
d) Basin no.2:

Distribution of **177** different individuals gradually hatched from **455** full eggs of *Galerucella nymphaeae* collected on **2.7.2012**



e) Basin no.2:

Distribution of **264** different individuals gradually hatched from **399** full eggs of *Galerucella nymphaeae* collected on **7.8.2012**



f) Basin no.2:

Distribution of **133** different individuals gradually hatched from **215** full eggs of *Galerucella nymphaeae* collected on **23.8.2012**

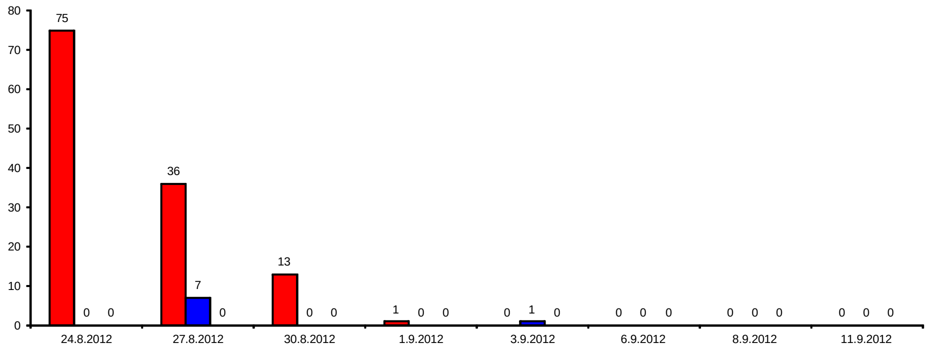


Fig. 9. Successive hatching of larvae of *Galerucella nymphaeae* and imagoes of *Oomyzus gallerucae* and *Aprostocetus celtidis* from full eggs of *G. nymphaeae* collected in 3 terms in two basins with water lilies (*Nymphaea* sp.) in BG PJŠU.

chalcid wasps of *Aprostocetus celtidis* species (Erdös, 1954) (Hymenoptera: Eulophidae: Tetrastichinae) (Fig. 8) occured. The course of hatching of leaf beetle larvae as well as adult parasitoids (on the basis of continuous sample checking) is shown in graphs (Fig. 9). The summary data for all collected *G. nymphaea* eggs is in Tab. 1.

Considering the hatching of individuals of different species within the individual layings (egg clutches) of *G. nymphaeae*, no individuals were registered from 138 egg clutches (eggs empty upon collecting, damaged prior to collecting or possibly later on due to unfavourable conditions in the vials), 104 egg clutches gave rise solely to leaf beetle larvae, 23 clutches gave rise only to chalcid wasps (18 clutches – solely *O. gallerucae*, 2 clutches – solely *A.celtidis*, 3 clutches - *O.gallerucae* + *A. celtidis*) and only in four cases the same egg clutch gave rise to mixture of leaf beetle larvae and chalcid wasp adults (*O. gallerucae*) (though with an interval between them within the hatching process). The example of distribution of hatching individuals from individual egg clutches is shown in Tab. 2 (3rd sampling, 23rd July 2012, from Basin no.1).

During the first visit to natural habitat near Klin nad Bodrogom, the occurrence of leaf beetle *G. nymphaeae* was relatively rare in spite of local massive presence of its host plants (*N. alba*, and mainly *N. lutea*). Though with much effort only 177 eggs were collected (40 of them already hatched - empty), 31 older larvae and 47 pupae. No parasitoids hatched from the collected eggs even after a longer time period. Eleven days after this sampling, 10 adults of *A. lucens* hatched from one pupa (1 ♂ and 9 ♀♀) (Fig. 10). After 21 days from the date of sampling one chalcid wasp (♀) of the above mentioned species was found from one of the collected larvae. After 28 days from the date of sampling six chalcid wasps of *Tetrastichus clito* (Walker, 1840) (Hymenoptera: Eulophidae: Tetrastichinae) (1 ♂ and 5 ♀♀) (Fig. 11) hatched from one pupa of *G.nymphaeae* collected in the field.

Discussion

From the faunistic point of view, all the mentioned identified species of chalcid wasps (*O.gallerucae*, *A. celtidis*, *A. lucens* and *T. clito*) represent the first ever registered occurrence in Slovakia. However, their detected association with *G. nymphaeae* is even more interesting, as so far only *A. lucens* from them was referred to in literary sources to be the parasitoid of this leaf beetle. According to NOYES (2012) the following host spectrum has been known for individual species (using the valid names and exclusion of synonyms also in hosts) up to now:

O. gallerucae: *Cassida rubiginosa* O.F. Müller, 1776 (on *Cirsium* sp.), *Galerucella lineola* (Fabricius, 1781) (it can feed on Betulaceae, Corylaceae and Salicaceae), *Galerucella singhara* Lefroy (on *Trapa* sp.), *Xanthogaleruca luteola* (O.F. Müller, 1776) (on *Ulmus* sp.),

A. celtidis: *Xanthogaleruca luteola* (on *Ulmus* sp.), *Pyrrhalta viburni* (Paykull, 1799) (on *Viburnum* sp.) and *Phyllonorycter lantanella* (Schrank, 1802) (on *Viburnum* sp. and *Sorbus* sp.)

A. lucens: *G. nymphaeae* (on Nymphaeaceae), *Lochmaea suturalis* (C.G. Thomson, 1866) (on *Calluna* sp.)

T. clito: *Cassida deflorata* Suffrian, 1844 (on Asteraceae), *Cassida murraea* Linnaeus, 1767 (on Asteraceae), *Cassida nebulosa* Linnaeus, 1758 (on Chenopodiaceae), *C. rubiginosa* (on *Cirsium* sp.), *Cassida viridis* Linnaeus, 1758 (on Lamiaceae)

Other registered associations for *A. lucens* are mentioned by HAMBÄCK et al. (2007), there are following chrysomelid leaf beetles: *Galerucella tenella* (Linnaeus, 1761) on *Filipendula ulmaria* (Rosaceae) and *Galerucella calmariensis* (Linnaeus, 1767) on *Lythrum salicaria* (Lythraceae).

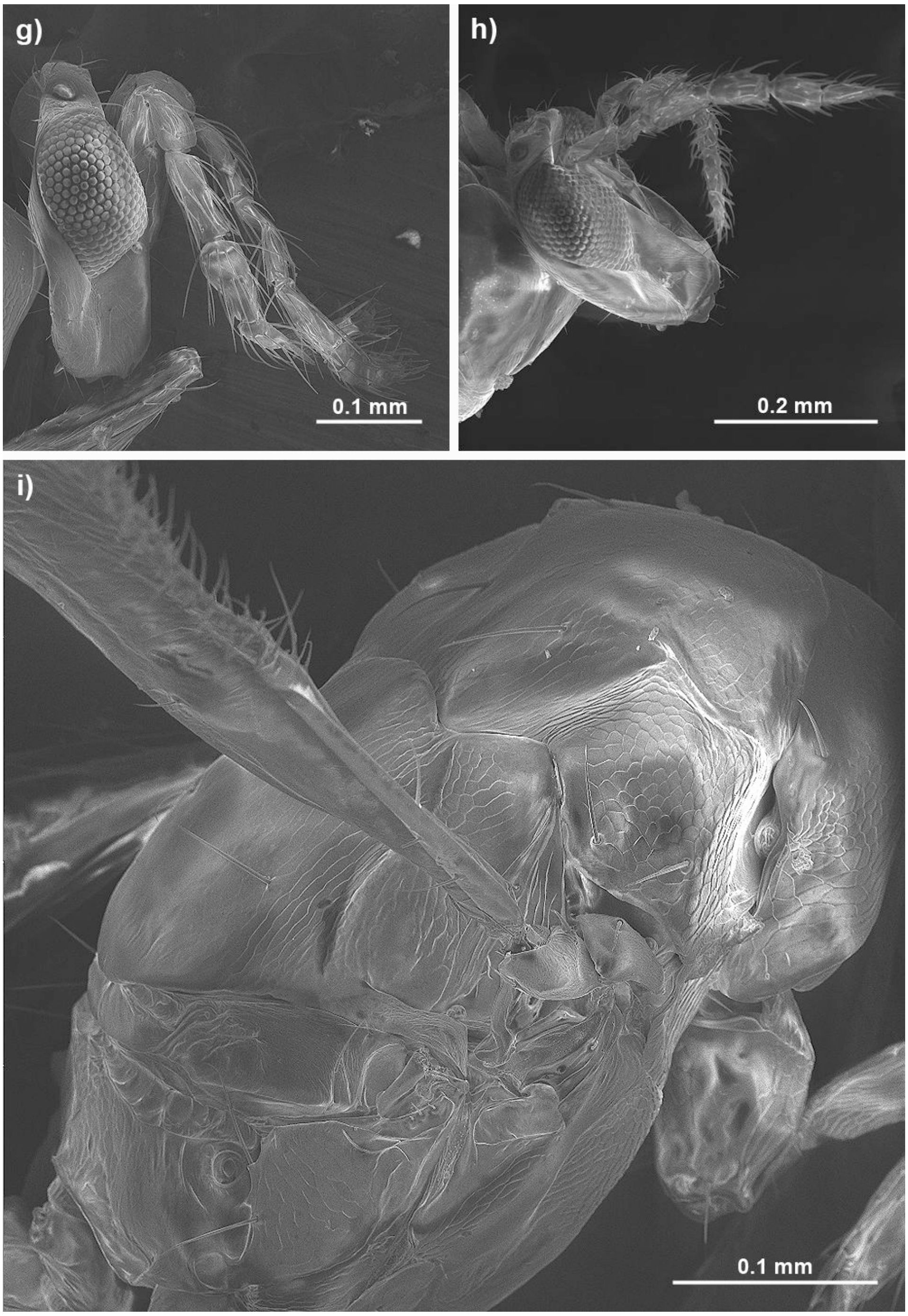
The survey of associations above shows that the known hosts of ***O. gallerucae*** are the representatives of Chrysomelidae family. With regard to the environment, only the case of *G. singhara* feeding on water plants *Trapa* sp. (Lythraceae) ecollogically resembles our findings though geographically it is a distant region (Asia) and different family of host plants. Although *O. gallerucae* is known to occur in Europe, Asia, Northern and Southern America (NOYES 2012) where the occurrence of both the representatives of Nymphaeaceae family and the related phytophagous *Galerucella* beetles are frequent, our findings about the association of this chalcid wasp to *G. nymphaeae* represent the first ever record of such parasitation. The average value of individual parasitized eggs was not very high (11.6 %), maximum value (29.4 %) was determined from the first set of samples in Basin no.1. On the other hand, the results of hatching within individual *G. nymphaeae* egg clutches suggest that the female of chalcid wasp *O. gallerucae* when laying its own eggs usually attacks all the laid eggs of the leaf beetle in a clutch (there were, in total, only 4 cases of concurrent hatchings of leaf beetle larvae and chalcid wasp adults within the individual egg clutches).

In the case of ***A. celtidis*** none of until now published records referred to water plants environment. Among the above mentioned hosts (NOYES 2012) there were identified not only beetles from Chrysomelidae family but also a moth species (*P. lantanella*) from Gracillariidae family and in all the cases these are referred to as primary hosts. Our results, however, indirectly suggest the possibility of hyperparasitism where *A. celtidis* attacks eggs of *G. nymphaeae* probably already parasitized by *O. gallerucae*. Chalcid wasps of *A. celtidis* only occurred from one collection of samples (Tab. 2) where hatching of various species (leaf beetle larvae and chalcid wasp adults) occurred within 36 egg clutches of *G. nymphaeae*. The individuals of *O. gallerucae* were registered from 10 egg clutches and individuals of *A. celtidis* originated from 5 clutches. In 3 of these cases the adults of both species of chalcid wasps - *O. gallerucae* and *A. celtidis* hatched from the same egg clutch of water-lily beetle. Hypothetically, considering accidental co-occurrence in an egg clutch, in simplified model with total 36 egg clutches and 10 of them attacked by *O. gallerucae*, there is only 10.3 % probability that within 5 egg clutches accidentally selected by females of *A. celtidis* just 3 egg clutches would have already been attacked by *O.gallerucae*

Fig. 10. ***Asecodes lucens*.**

pupa of *Galerucella nymphaeae* (from which 10 individuals of *A. lucens* hatched): a); females of *A. lucens*: b) – f), h), i); male of *A. lucens*: g);

b) – body in ventro-lateral view; c) – body in lateral view; d) – body in dorsal view; e) – body in frontal view; f) – right fore wing from above; g), h) – lateral views of heads; i) – caudo-dorso-lateral view of mesosoma.


g)
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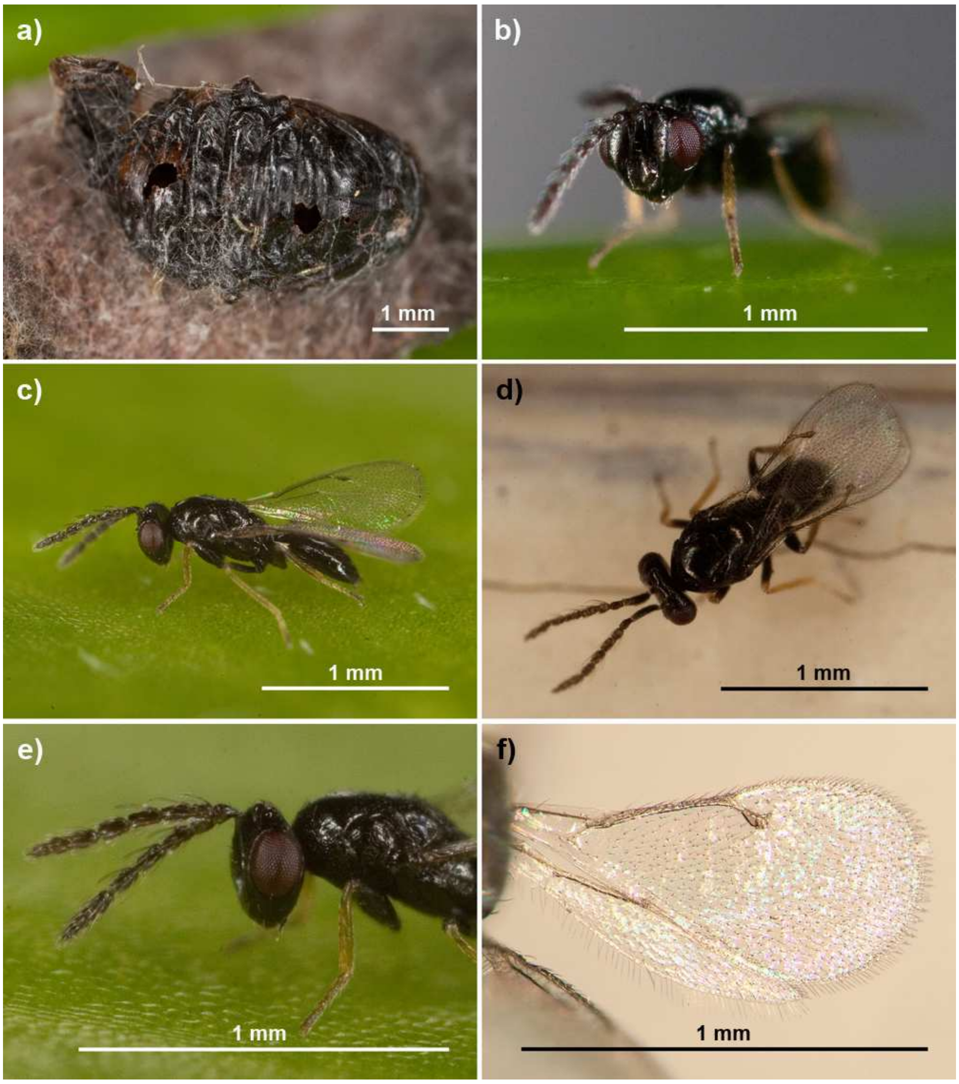
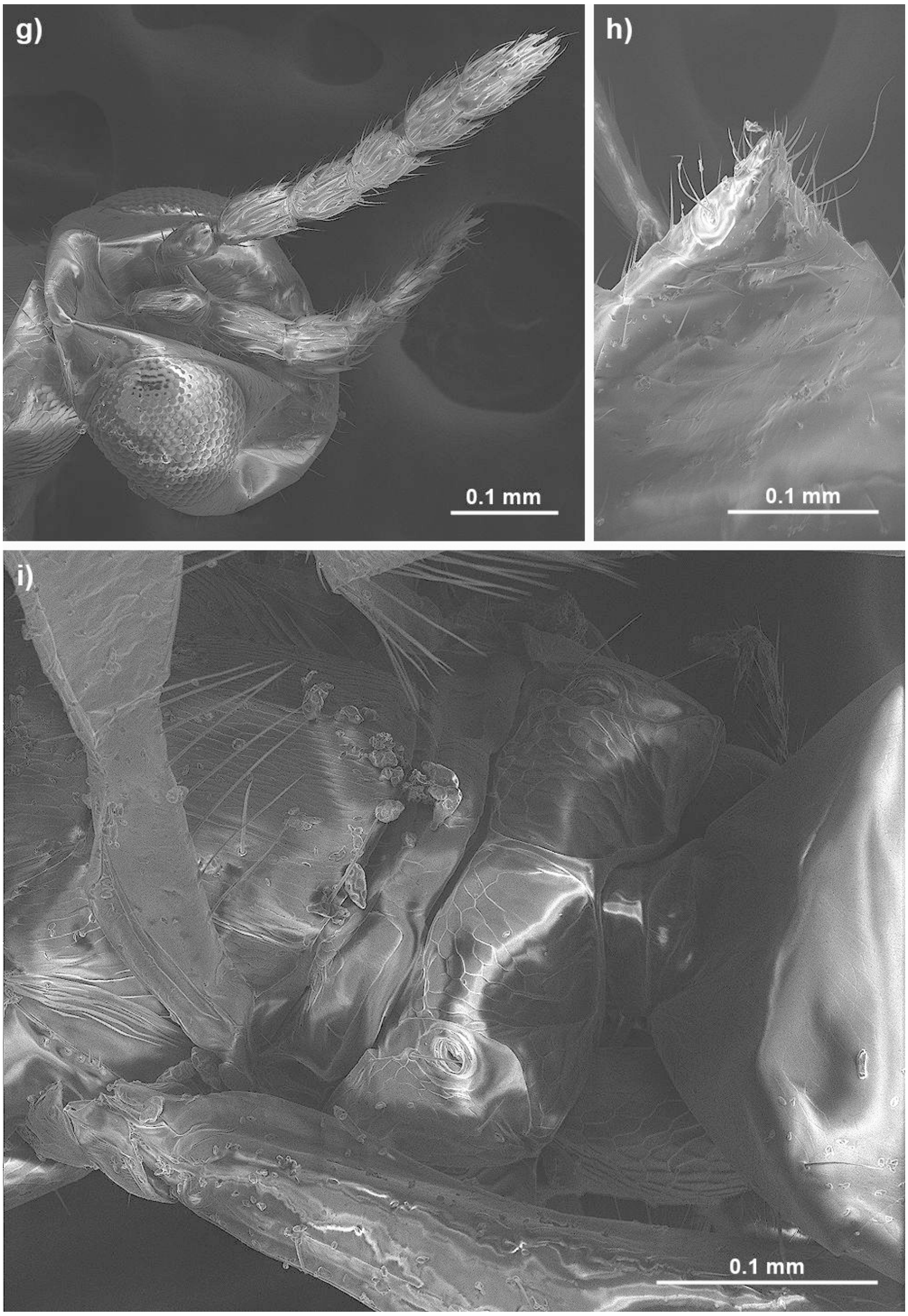


Fig. 11. *Tetrastichus clito*.

pupa of *Galerucella nymphaeae* (from which 6 individuals of *T. clito* hatched): a);
male of *T. clito*: b) – e);
females of *T. clito*: f) – i);

b) – body in frontal view; c) – body in lateral view; d) – body in dorsal view; e) – lateral view of head and antennae; f) – right fore wing from above; g) – fronto-lateral view of head; h) – tip of abdomen and cercal setae from above; i) – caudo-dorso-lateral view of scutellum, metanotum and propodeum.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Tab. 1. Summary data on different individuals (larvae of *Galerucella nymphaeae* and adult chalcid wasps of *Oomyzus gallerucae* and *Aprostocetus celtidis*) hatched from eggs of *G. nymphaeae* collected in 3 terms (2.7.2012, 7.8.2018 and 23.8.2012) in two basins (Basin no. 1 and Basin no. 2) with water lilies (*Nymphaea* sp.) in BG PJŠU.

Numbers (n) of collected eggs and individuals hatched from eggs	Basin no. 1								Basin no. 2								Total	
	Dates of egg sampling						Total		Dates of egg sampling						Total			
	2.7.2012		7.8.2012		23.8.2012				2.7.2012		7.8.2012		23.8.2012					
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
all collected eggs	482		347		949		1778		551		705		412		1668		3446	
empty eggs (hatched before sampling)	169		161		538		868		96		306		197		599		1467	
full eggs (in time of sampling)	313	100.0	186	100.0	411	100.0	910	100.0	455	100.0	399	100.0	215	100.0	1069	100.0	1979	100.0
all observed individuals hatched from eggs	133	42.5	122	65.6	276	67.2	531	58.4	177	38.9	264	66.2	133	61.9	574	53.7	1105	55.8
larvae of *G. nymphaeae*	41	13.1	111	59.7	220	53.5	372	40.9	105	23.1	257	64.4	125	58.1	487	45.6	859	43.4
all imagoes of chalcid wasps	92	29.4	11	5.9	56	13.6	159	17.5	72	15.8	7	1.8	8	3.7	87	8.1	246	12.4
O. gallerucae (in total)	92	29.4	11	5.9	39	9.5	142	15.6	72	15.8	7	1.8	8	3.7	87	8.1	229	11.6
♂♂	33	10.5	4	2.2	8	1.9	45	4.9	22	4.8	1	0.3	1	0.5	24	2.2	69	3.5
♀♀	59	18.8	7	3.8	31	7.5	97	10.7	50	11.0	6	1.5	7	3.3	63	5.9	160	8.1
A. celtidis (in total)	0	0.0	0	0.0	17	4.1	17	1.9	0	0.0	0	0.0	0	0.0	0	0.0	17	0.9
♂♂	0	0.0	0	0.0	3	0.7	3	0.3	0	0.0	0	0.0	0	0.0	0	0.0	3	0.2
♀♀	0	0.0	0	0.0	14	3.4	14	1.5	0	0.0	0	0.0	0	0.0	0	0.0	14	0.7
remaining unhatched eggs	180	57.5	64	34.4	135	32.8	379	41.6	278	61.1	135	33.8	82	38.1	495	46.3	874	44.2

Tab. 2. Numbers of individuals of *G. nymphaeae* larvae (red) and adult chalcid wasps (*O. gallerucae* (blue) and *A. celtidis* (green)) hatched from individual egg clutches of *G. nymphaeae* collected from Basin no. 1 on 23rd August 2012.
Only the egg clutches with hatched individuals are listed - 36 out of total (82) collected clutches in this sampling event.

Numbers of collected eggs and individuals hatched from eggs	Codes of individual egg clutches collected 23.8.2012 in Basin no. 1 (egg clutches with no observed hatched individuals are not presented)																																				Total
	X01	X02	X03	X04	X05	X07	X10	X14	X15	X16	X19	X22	X23	X24	X27	X28	X29	X31	X32	X34	X37	X39	X49	X50	X55	X62	X67	X68	X72	X74	X76	X77	X78	X80	X81	X82	
all eggs in the respective clutch	14	6	14	8	9	14	10	10	16	15	10	7	10	13	16	8	11	10	16	16	9	11	11	12	12	16	13	15	10	12	8	11	11	9	9	12	414
empty eggs (hatched before sampling)	0	5	9	2	0	0	0	1	4	0	8	0	7	2	3	2	0	0	6	0	0	0	0	0	0	6	12	1	2	9	0	0	0	0	0	0	79
full eggs (in time of sampling)	14	1	5	6	9	14	10	9	12	15	2	7	3	11	13	6	11	10	10	16	9	11	11	12	12	10	1	14	8	3	8	11	11	9	9	12	335
all observed individuals hatched from eggs	12	1	3	6	7	13	10	9	12	2	1	5	3	9	13	4	7	7	10	14	7	10	11	12	9	9	1	13	5	1	8	10	6	8	9	9	276
larvae of *G. nymphaeae*	12	0	0	0	7	13	0	9	12	2	1	0	0	9	13	4	7	7	10	14	7	7	11	12	9	9	0	13	0	0	0	0	6	8	9	9	220
imagoes of chalcids (in total)	0	1	3	6	0	0	10	0	0	0	0	5	3	0	0	0	0	0	0	0	0	3	0	0	0	0	1	0	5	1	8	10	0	0	0	0	56
O. gallerucae (in total)		1	3				10					2	2									3					1		5		2	10					39
♂♂		0	2				1					0	0									1					1		0		1	2					8
♀♀		1	1				9					2	2									2					0		5		1	8					31
A. celtidis (in total)				6								3	1																	1	6						17
♂♂				3								0	0																	0	0						3
♀♀				3								3	1																	1	6						14
remaining unhatched eggs	2	0	2	0	2	1	0	0	0	13	1	2	0	2	0	2	4	3	0	2	2	1	0	0	3	1	0	1	3	2	0	1	5	1	0	3	59

(which would have left intact eggs within such clutch for later *A.celtidis* attack). From statistical viewpoint, and with the fact that females of *O. gallerucae* usually attack all eggs in a clutch, the direct selection of parasitized eggs by females of *A. celtidis* seems to be more probable explanation (it means hyperparasitism) than accidental co-occurrence. For completeness, the reverse order of attacks of both parasitoid species on eggs can be taken into account, or facultative hyperparasitic associations, etc. but, with regard to current data, these hypotheses seem to be less probable than hyperparasitism by *A. celtidis*. In the future, particular attention should be given to this indicated possibility of closer relations of both chalcid wasp species.

A. lucens detected in natural habitat near Klin nad Bodrogom was the only one which is mentioned in literary sources (usually referred to as *A. mento*) as the parasitoid of leaf beetle *G. nymphaeae* (summarised by NOYES 2012). It is also known for its association with other related species of *Galerucella* genus and is presented in several research works focused on more detailed analyses of host-parasitoid interactions. HAMBÄCK et al. (2006) show that *A. lucens* can be shared by two leaf beetle species (*G. calmariensis* and *G. pusilla*) in one place. The different selectivity of this parasitoid for individual beetle host species (with other community parameters) can result in interesting indirect interactions of associated host plants in mixed populations (STENBERG et al. 2007). It seems that just *A. lucens* could have the potential to increase the control rate in biological regulation of water-lily beetles in BG PJŠU.

Chalcid wasp ***T. clito*** has so far been known to be associated with the leaf beetles of *Cassida* genus. Hatching of adults from *G. nymphaeae* pupa represents the first ever record of such a relation not only for this water-lily beetle species but even within *Galerucella* genus. Yet it was a rare finding in the natural habitat of the dead branch of the river Bodrog. In the next seasons more material should be collected to consider the importance of this chalcid wasp in the spectrum of parasitoids there occuring.

From the viewpoint of practical protection of water-lily cultivars grown, the chalcid wasp *O. gallerucae* has not shown very significant impact on *G. nymphaeae* population in BG PJŠU. The lower rate of parasitation in later samplings could also be due to the unwanted effect of removing the major part of parasitoid population in the removed or crushed eggs of leaf beetles during sanitation measures. In the previous years the manual reduction of leaf beetle eggs could have been of significant importance, too. But even in times when such measures were not applied, the population of these beetles had very destructive impact on the leaves of water-lilies grown in the basins. It is not clear yet where *O. gallerucae* attacking the eggs of the leaf beetle in BG PJŠU comes from and since when it has been present there. However, this chalcid wasp alone is not able to reduce the damage of water lilies leaves to acceptable level yet. That is why the use of other possible parasitoids is considered to be applied in BG PJŠU, the most suitable candidate seems to be *A. lucens*. Prior to this, the spectrum of related leaf beetles on the vegetation surrounding the basins will have to be inspected, as well as the spectrum of their parasitoids (or eventually hyperparasitoids). The circumstances of mutual influence of existing, or possibly introduced, parasitoids in multi-trophic system of related types of leaf beetles and their natural enemies should be researched further on (HAMBÄCK et al. 2006, STENBERG et al. 2007). Besides parasitoids, the role of predators in the discussed water elements of BG PJŠU should be also reviewed more carefully.

Of course, information on ecology of these insect species related to aquatic macrophytes can be used in different way in general. In situations where some water lilies are considered to be weeds, the leaf beetles (and eventually hyperparasitoids) should be supported and their natural enemies, including the mentioned primary parasitoids, are unwanted. On the other hand, there is just the opposite situation for water-lily growers (like in BG PJŠU) with their aim to maximally reduce the damage to cultivated plants through suppression of leaf beetles as insect pests. Hyperparasitoids are undesired in this case, while primary parasitoids should be supported to attack especially the selected water-lily beetle species. Along with it, the attention should be paid to other available alternative hosts (seemingly indifferent from the viewpoint of target plant taxa cultivation) suitable for reproduction of the required natural enemies.

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References

BORGHUIS A., GROENENDAEL J. VAN, MADSEN O. & OUBORG J. (2009): Phylogenetic analyses of the leaf beetle genus *Galerucella*: Evidence for host switching at speciation? Molecular Phylogenetics and Evolution 53: 361–367.

CRONIN G., SCHLACHER T., LODGE D.M. & SISKA E.L. (1999): Intraspecific variation in feeding preference and performance of *Galerucella nymphaeae* (Chrysomelidae: Coleoptera) on aquatic macrophytes. J.N. Am. Benthol. Soc. 18, 391–405.

DING J. & BLOSSEY B. (2005): Invertebrate predation on the water lily beetle, *Galerucella nymphaeae* (Coleoptera: Chrysomelidae), and its implications for potential biological control of water chestnut, *Trapa natans*. Biological Control 35: 17–26.

GRAHAM M.W.R. DE V. (1987): A reclassification of the European Tetrastichinae (Hymenoptera: Eulophidae), with a revision of certain genera. Bulletin of the British Museum (Natural History) (Entomology) 55(1) pp. 1-392.

GRAHAM M.W.R. DE V. (1991): A reclassification of the European Tetrastichinae (Hymenoptera: Eulophidae): revision of the remaining genera. Memoirs of the American Entomological Institute No 49 pp. 322 pp.

GREGOREK R. (2011): Influence of intensive fertilization on growth performance of decorative cultivars of *Nymphaea* sp. in Botanical Garden of P.J.Šafárik University in Košice, Slovakia. Thaiszia – J. Bot. 21: 123-130.

HAMBÄCK P.A., STENBERG J.A. & ERICSON L. (2006): Asymmetric indirect interactions mediated by a shared parasitoid: connecting species traits and local distribution patterns for two chrysomelid beetles. Oecologia 148: 475–481.

NESTEROVA O. (2008): Towards the morphology and biology of preimaginal stages of sibling-species of genus *Galerucella* Crotch. Research on Chrysomelidae. The Netherlands. 2008. Roc 1. P. 121-128, 379-380.
NOKKALA CH., NOKKALA S. & NORDELL-PAAVOLA A. (1998): European and North American populations of *Galerucella nymphaeae* (Coleoptera: Chrysomelidae): Two separate species revealed by chorion polypeptide analysis. Eur. J. Entomol., 95: 269-274.
NOYES J.S. (2012): Universal Chalcidoidea Database. World Wide Web electronic publication. http://www.nhm.ac.uk/chalcidoids [accessed 12 Dec. 2012]
SCHLACHER T.A. & CRONIN G. (2007): A trophic cascade in a macrophyte-based food web at the land–water ecotone. Ecological Research 22: 749-755.
STENBERG J.A., HEIJARI J., HOLOPAINEN J.K. & ERICSON L. (2007): Presence of *Lythrum salicaria* enhances the bodyguard effects of the parasitoid *Asecodes mento* for *Filipendula ulmaria*. Oikos 116: 482-490.
STENBERG J.A. & STENBERG J.E. (2012): Herbivory limits the yellow water lily in an overgrown lake and in flowing water. Hydrobiologia 691:81–88.
TRJAPITZIN V.A. (1978): Hymenoptera. II., Chalcidoidea, 13. Eulophidae (excl. Tetrastichinae). Pp. 381-487 In: MEDVEDEV, G. S. (Ed.): Opredeliteli Nasekomykh Evropeyskoy Chasti SSR, T. III. Pereponchatokrylye. Ch. 2, Nauka, Leningrad, 757 pp.
WARCHAŁOWSKI A. (2003): Chrysomelidae. The Leaf-Beetles of Europe and the Mediterranean Area. Natura Optima Dux Foundation, Warszawa, Poland, 600 pp.

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