Complex of new species for Romanian insect fauna involved in natural control of annual ragweed, *Ambrosia artemisiifolia* L. (Asterales: Asteraceae)

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Abstract: This study provides data on the first known established populations of some alien species in Romania: *Ophraella communa* LeSage, 1986 (*Coleoptera: Chrysomelidae*), *Acontia candefacta* (Hübner, 1831) (*Lepidoptera: Noctuidae*), and *Peridroma saucia* (Hübner, 1808) (*Lepidoptera: Noctuidae*). The study marked the first identification of the species *Adaina ambrosiae* (Murtfeldt, 1880) (*Lepidoptera: Pterophoridae*), *Nysius ericae ericae* (Schilling, 1829), *N. huttoni* (White, 1878), *N. cymoides* (Spinola, 1837) and *N. helveticus* (Herrich-Schaeffer, 1850) *(Heteroptera: Lygaeidae),* as part of the Romanian insect fauna. Additionally, the study provides a detailed analysis of the distribution and agricultural impact of expanding the existing knowledge about this species.

The paper also presents the current distribution of these species and briefly discusses their presence in specific areas of Romania. Further studies will be necessary to determine the range of host species in the new habitat conditions and to evaluate any potential economic impact on other hosts from the *Asteraceae* family, such as sunflower.

Key-words: *Ambrosia artemisiifolia, biological control, Adaina ambrosiae, Ophraella communa, Nysius species*

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1 Introduction

Ambrosia artemisiifolia is an alien invasive species in Romania, included in the IAPs List (EPPO List of Invasive Alien Plants), with EPPO status A2 for grasslands. He is native to the North American continent. In Europe, it was identified for the first time in 1863, in Germany, namely in the specific spreading area of Brandenburg and Pfaffendorf, [42]. Now, it is spread worldwide except in the northern part of Russia and more parts of Africa. The Ambrosia genus includes 36 species and 4 subspecies native to North America. However, some of these species have been expanded their range and become invasive worldwide [32, 33]. *A. artemisiifolia* is an annual Asteraceae, which has invaded ranges in every continent except Antarctica [33]. Two of the species of the genus are the most dangerous and with a high range of invasion: common ragweed, *A. artemisiifolia,* and great ragweed, giant ragweed *A. trifida* L. In its native range, *A. artemisiifolia* grows best under temperate, continental climates [15].

In Romania, *A. artemisiifolia* was first recorded in 1908, at the Orşova railway station, Mehedinţi County, at the time part of the Kingdom of Austria-Hungary [83]. Romania ranks among the European countries most affected by Ambrosia, which poses a significant threat to public health and the environment, thus generating considerable public concern. The actual status in Romania is that it is considered an invasive species, accidentally introduced, and indicated from all the provinces of the country, but its spread is still incompletely known [83]. Common ragweed is mentioned in literature as an invasive weed that is commonly found in disturbed sites such as roadsides, railways, gravel pits, construction sites, agricultural land, waterways, etc. The greater incidence of this species is in the western counties (Timiş, Arad, and Bihor) [43, 80]. It was initially documented between 1907 and 1912 in the Banat region, and it has since become prevalent across Romania [31].

Efforts to manage *A. artemisiifolia* have included biological control programs. While nearly 450 insect, mite, or fungus species have been reported associated with Ambrosia plants, most are polyphagous and do not significantly affect common ragweed. Effective biocontrol relies on long-term biocontrol agents like *Zygogramma suturalis* and *Epiblema strenuana*) [39, 25]. In its native range, common ragweed is subjected to a variety of invertebrate herbivores, including the beetles *Z. suturalis* F., *O. communa* (Coleoptera: Chrysomelidae), the moth *E. strenuana* (Lepidoptera: Tortricidae), and others, as well as

polyphagous consumers such as beetle, moth, and bug feeding species [53]. Concerning the pathogens, Farr reported more than 25 fungi that infect common ragweed [36]. Studies have already been conducted in Romania on the use of local or imported natural enemies in areas already infested with ragweed, inspired by programs targeting the Colorado potato beetle, starting around 1992-1993.

This study presents the current distribution of these species and briefly discusses their presence in specific areas of Romania.

2 Materials and Methods

The selected habitats, mainly grasslands, must allow for the existence of precise location records of the *A. artemisiifolia* populations. Surveys of suitable sites, such as construction sites, roadsides, cultivated lands, and areas with ruderal vegetation, to find populations of insects that were could used like natural enemies of common ragweed. We recorded insect species found on the plants, their life stage, damage symptoms, and phenology of the plants being attacked. The biological samples of collected arthropods were kept alive separately (for mass rearing in laboratory conditions) and others were preserved in special cages with 90% ethyl alcohol. The surveys started in 2016, targeting spring (April-May) and late summer (August-September) in 22 districts where plant hosts were developed in relatively large areas and present in years from year to year, representing almost the whole country (Table 1)

Table 1

District	Localities
Timiș	Lugoj, Giroc, Jimbolia, Recaș, Giarmata, Dumbrăvița
Arad	Rîmeț, Teiuș, Moneasa, Gurahonț, Slatna, Dezna, Săvîrșin, Chișineu-Criș, Vinga, Miniș, Pecica, Șiria, Pînacota, Lipova, Ghioroc, Nădlac, Ineu, Macea, Curtici, Sîntana
Alba	Alba Iulia, Blaj, Sebeș
Olt	Caracal, Balș, Slatina, Osica de Sus, Drăgănești-Olt
Sălaj	Zalău, Crasna, Boghiș, Nușfalău, Șimeul Sivaniei
Ilfov	București, Buftea, Snagov, Corbeanca, Mogoșoaia, Chiajna, Bragadiru, Dobroești
Mehedinți	Balota, Drobeta Turnu-Severin, Orșova, Șvinița, Baia de Aramă, Isverna
Dîmbovița	Tîrgoviște, Dîrmănești, Aninoasa, Răcari, Lungulețu, Tărtășești, Ciocănești, Samurcași
Vrancea	Focșani, Cotești, Golești, Odobești, Soveja, Paltin, Rîmnicu Sărat
Vîlcea	Băbeni, Rîmnicu Vîlcea, Olănești, Govora, Călimănești, Horezu, Cozia, Voineasa, Obîrșia Lotrului
Argeș	Pitești, Călinești, Leordeni, Curtea de Argeș, Rucăr, Cîmpulung, Poenari
Gorj	Tîrgu Jiu, Tismana, Sohodol, Hobița, Padeș, Runcu, Curtișoara
Prahova	Vălenii de Munte, Slănic, Ploiești, Urlati, Valea Călugarească, Băicoi, Cîmpina, Comarnic, Breaza, Sinaia, Posada, Nistorești, Pietriceaua, Poiana-Țapului, Bușteni, Cheia
Bihor	Oradea, Beiuș, Roșia, Pietroasa, Nucet, Izbuc
Satu Mare	Satu Mare, Ardud, Carei, Livada, Certeze, Negrești-Oaș, Halmeu
Cluj	Turda, Gherla, Dej, Huedin, Beliș, Gilău
Valsui	Solești, Vaslui, Moreni, Bîrlad, Zorleni, Tutova
Galați	Galați, Costache Negri, Tecuci, Movileni, Valea Mărului, Tîrgu Bujor, Smîrdan, Independența, Tudor Vladimirescu
Dolj	Craiova, Podari, Segarcea, Plenița, Băilești, Moțăței, Coșoveni, Malu Mare, Pielești, Șimnicu de Jos, Mischii, Işalnița, Filiași, Beharca
Caras-Severin	Oravița, Anina, Moldova Nouă, Băile Herculane, Teregova, Armeniș, Slatina-Timiș, Caransebeș, Văliug
Bacău	Răcăciuni, Letea-Veche, Moinești, Tescani, Tîrgu Ocna, Onești, Borzești, Slănic-Moldova, Oituz, Buhuși, Hemeiuș, Lilieci
Sibiu	Ocna Sibiului, Axente-Sever, Copșa Mică, Mediaș, Micăsasa, Sălișteː, Cristian, Apoldu De Jos, Poplaca, Șelimbăr, Rășinari, Cisnădie, Avrig, Porumbacu de Jos, Sîmbăta de Sus

In every district, the localities were chosen according to the information obtained from the local authorities (mayors, phytosanitary centers, and some informed citizens) (Appendix 1).

1. Taking the qualitative samples (Direct collection method)

The *A. artemisiifolia* plants were observed individually in connection with all the arthropods involved in some actions (resting on the plant, or feeding by stinging and sucking or eating leaves or buds, shoots, and inflorescences) and some individuals involved in biological control like natural enemies were collected dead or alive. The predatory arthropod species were only noted trying to identify them as much as possible.

2. Belt method

Netting was conducted inside and outside areas with Ambrosia present throughout the entire vegetation period, using an entomological net measuring 0.30 m in diameter and 1 m in length. Each sample consisted of all insects collected over an area of 100 meters in length and 1 meter in

width. The number of samples (or walks) varied depending on the size of the habitat, ranging from 1 to 5. Host plants were first shaken into plastic bags to collect biological material, which was then transferred into special cages, preserved in 90% ethyl alcohol, and transported to the laboratory for taxonomic identification. Additional samples were collected by sweeping the net across a 100-meter distance, with the fauna collected representing an area of 100 square meters [52].

3. UV light traps method

The UV light traps were installed in different Plant Protection Inspection Services from the Romanian districts. The UV light source was placed at 3.5 -6 m above the surface of the ground. A sample consisted of insects collected over 72 hours [52].Identifying the species within the family Lygaeidae, particularly those of the genus Nysius, was challenging due to their small size, behavioral variability, and significant morphological similarities among closely related species. To overcome these difficulties, we employed a comprehensive approach that included consulting various bibliographic sources. These sources provided taxonomic keys, morphological descriptions, and analyses of male and female genital structures, which were especially valuable when comparative material was available.We were relying on key taxonomic studies and identification keys e.g., [9,12,10,20,21,29,34,46,59,69,70,71,89]. Additional references were used for detailed species descriptions, geographic distributions, host plants, and supplementary materials such as photographs and drawings [9,13,11,14,17,63,66,85,97,100].

3 Results and Discussions

Our investigations covered 91 localities across 22 counties in Romania, focusing on the distribution and potential natural enemies of *A. artemisiifolia*. Distribution of habitats where *A. artemisiifolia* and its associated arthropod fauna were identified (Fig.1).

A total of 4,550 arthropods were collected, predominantly insects and spiders from the order Araneae.

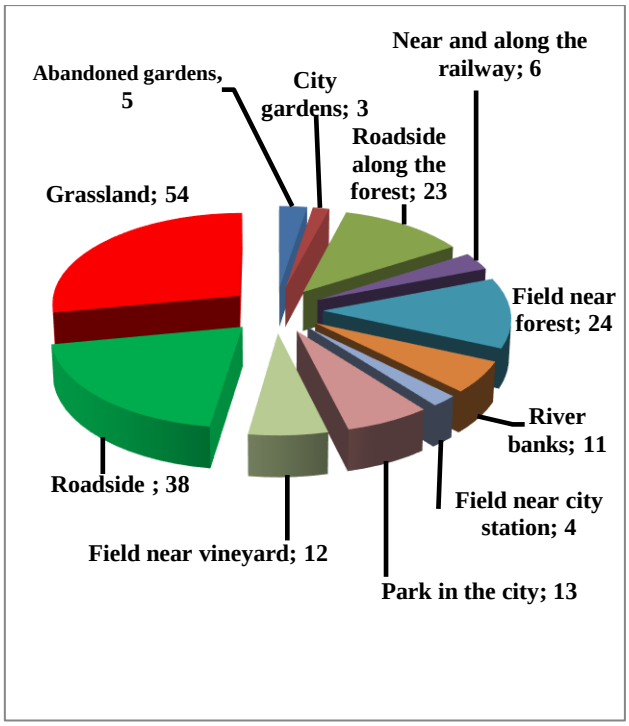


Fig. 1 – Type of the habitat invaded by *Ambrosia artemisiifolia* investigated

The true bugs guild was the most abundant with 18 families, 103 genus and 135 species (a total of 2,492 individuals) (Fig. 2).

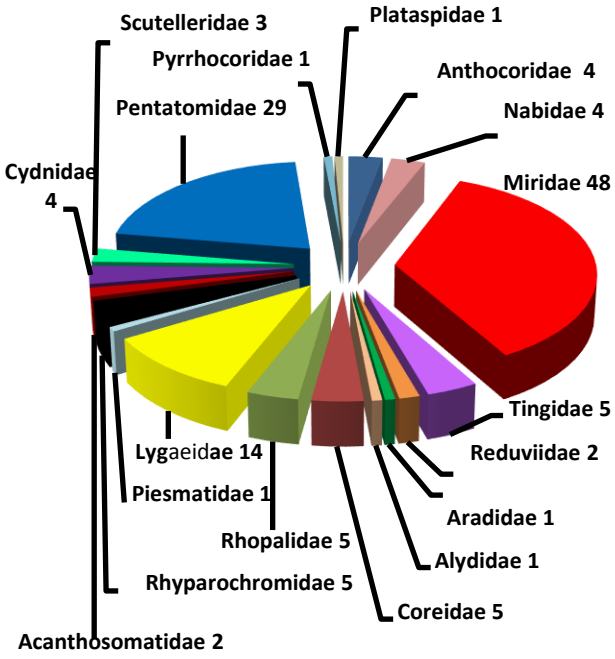


Fig. 2 – Structure of bug families collected on *Ambrosia artemisiifolia* habitats

Phytophagous species were the most abundant 109, zoophagous 9, and zoophytophagous 4 species, respectively (Fig. 3) (Appendix 2).

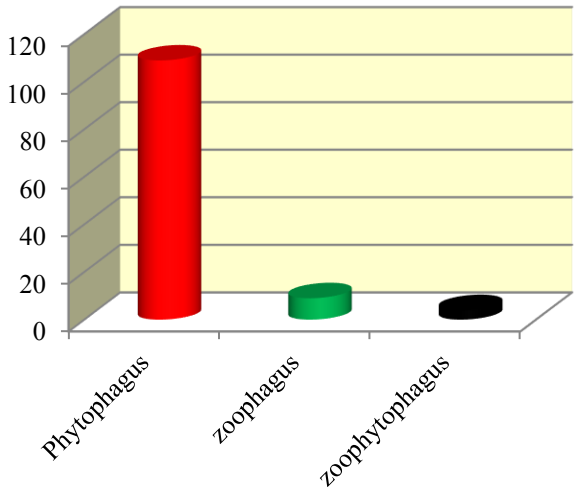


Fig. 3 – Trophyc range of guild bug species on *Ambrosia artemisiifolia* L.

From phytophagous many species were generalists with a wide range of host plants; therefore, it was difficult to determine their trophic preferences in detail. However, we could observe on the field that many of the species, especially from Miridae family fed on the *A. artemisiifolia* inflorescences, meaning on Ambrosia seeds in the last three months of *A. artemisiifolia* vegetation (Fig. 4).

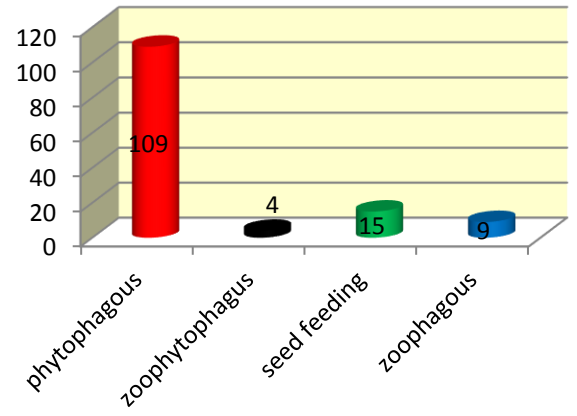


Fig. 4 - Useful species for the biological control of the invasive species *A. artemisiifolia*, seed feedings

Considering the huge seed production of this species, this trophic behavior can be useful in the natural biological control of the weed and the constant pressure of the weed reproduction could diminish their spreading in the area considered. Among the numerous bug species identified, four new Lygaeidae species for Romania were distinguished, observed feeding on *Ambrosia* seeds, and are presented below with brief descriptions.

Nysius huttoni (F.B. White, 1878) (Heteroptera: Lygaeidae)

N.huttoni is a species endemic to New Zealand and was recently accidentally introduced in Europe [37]. In New Zealand, *N.huttoni* is an economically important pest species, known as the wheat bug [85]. At present *N. huttoni* has been found in Europe (the Netherlands, Belgium, United Kingdom, and France) [11]. *Nysius huttoni* was probably introduced to the region through the international shipment of apple and kiwi fruits from New Zealand. Nonetheless, the precise location of its introduction remains unidentified, and the insect has not been detected during phytosanitary inspections in Belgium [17]. Laboratory studies revealed that 84.4% of adult *N. huttoni* and 36.8% of its eggs could endure conditions akin to those encountered during overseas transport (specifically, complete darkness at 0°C) for five weeks [17]. However, the conclusions on the potential spreading of this species across Europe could be assumed.

The species is similar morphologically to the group of *N. ericae* (Schilling, 1829) and *N. thymi* but can be easily distinguished by the long erect pubescence on pronotum, scutellum, corium, and clavus, the double row of punctures along the clavate suture, and in submacropters and brachypters also by the remarkably convex hemielytra [69]. Adults have a cryptic pattern in grey and/or brown with black and creamy white. Adults measured 2.4 to 4.5 mm in length, with an elongated, oval, and dorsally flattened body (short-winged forms appeared slightly convex), a triangular head slightly narrower than the pronotum, prominent non-stalked round eyes, antennal segments 1 and 4 broader than 2 and 3 (segment 4 being fusiform), and a labium extending to the hind coxae.

Plant species from the Brassica genus and Fabaceae (alfalfa, red and white clover) are attacked. In the origin habitat, no natural enemies are known apart from predation by an introduced species of bird (*Sturnus vulgaris* L.). *N. huttoni* overwinters as an adult and has two or three generations a year in New Zealand. The number of

generations in Europe is yet not clear because the studies on the species are just at the beginning but related species under the same climatic conditions have one or two generations per year. In Netherland and Belgium the species appears to feed on a range of wild plants and several observations support *N. huttoni* not being a threat to crops in Belgium under the present conditions of climate and soil usage. In our study we found *N. huttoni* feeding on the plant inflorescence in the second half of August when *A.artemisiifolia* is blooming. The insects are looking for fresh seeds exerting a strong pressure on the reproductive process of the species and could be considered an efficient biological control agent. This behavior suggests its potential as an effective biological control agent, warranting further studies to explore its application in managing *A. artemisiifolia* populations. The distribution in Romania is presented on the map (Fig. 5).

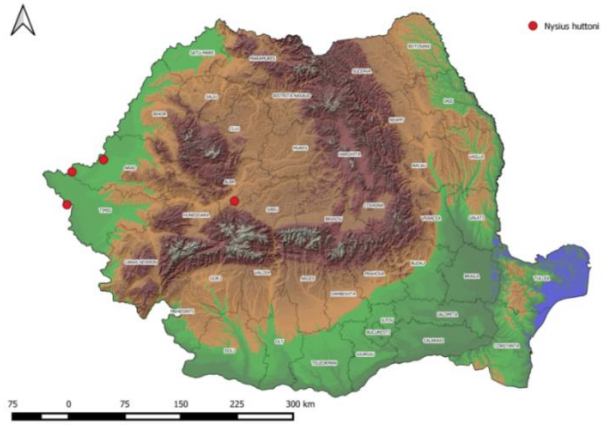


Fig. 5 - *Nysius huttoni* in Romania

Nysius helveticus (Herrich-Schaffer, 1850) (Heteroptera: Lygaeidae)

Synonyms: *Artheneis helveticus* (Herrich-Schaeffer,1850), *Cymus helveticu* Herrich-Schaeffer, 1850, *Cymus helveticus* Meyer-Dur, 1843, *Heterogaster lineatus* Costa, 1855, *Macroparius helveticus* (Herrich-Schaeffer, 1850), *M.s lineatus* (Costa), *N. lineatus* (Costa), *N. obsoletus* Fieber, 1861. The coloration of the body is typically flavescent or testaceous. *N.helveticus* can be identified distinctly from other species by its scutellum, which features a clearly defined flavescent carina, a corium that is almost translucent, and a lateral margin of the corium that is not markedly arched. It was mentioned in Spain [93,], Serbia [81], Turkey [103,10,24,66,23,103], R.Moldova [30], Russia [47,40,64], Iran [51], Mongolia [101], China [101]. *N.helveticus*, had fed on many host plants like polyphagous on *Achillea millefolium* L., (1753), *Anthemis tinctoria* (L.) J. Gay ex Guss, (1845) *Calluna vulgaris* Salisb., (1802), *Erica cinerea* L., (1753) *Filago arvensis* L.,(1753), *Hypericum perforatum* L., (1753), *Poa bulbosa* L., (1753), *Potentilla astracanica* Jacq., (1781), *Senecio vulgaris* L., (1753), *Tanacetum vulgare* L., (1753), etc. In the area where it was present, both nymphs and adults were observed feeding on fruits and certain vegetables.

In Romania in the area where had been observed another host plant outside common ragweed was *Poa pratensis* L., (1753), *Fraxinus excelsior* L., (1753), *Achillea millefolium* L., (1753), *Lythrum salicaria* L., (1753), *Lathyrus odoratus* L., (1753), and *Silene vulgaris* (Moench) Garcke, (1869) only feeding on flowers and seeds.

As with *N. huttoni*, the current distribution of this species has been mapped (Fig. 6) and should be considered in future studies regarding its economic impact on cultivated crops, such as wheat, and its potential role as a biological control agent against common ragweed.

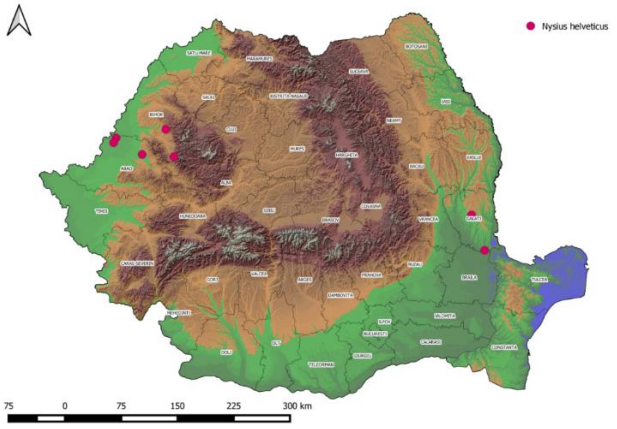


Fig. 6 – Map distribution of *Nysius helveticus* in Romania

Nysius ericae ericae (Schilling, 1829) (Heteroptera: Lygaeidae)

Synonyms: *Lygaeus thymi* Wolff, 1804, *Macroparius ericae* (Schilling, 1829), *N.s albidus* Dallas, 1852, *N. albidus* Jakovlev, 1867, *N. ericae* subsp. *Ribesi* Wagner, 1965, *N. gracilis* Scott, 1870, *N. maculatus* Fieber, 1861, *N. natalensis* Evans, 1929. The body color is yellowish brown. *N. ericae ericae* is very similar to *N. thymi* in coloration and body size. It is distinguished from the latter by the end of the buccula is sharpened or somewhat blunt, and the first segment of the rostrum commonly exceeds or is equal to the buccula. It was signaled in Montenegro [76, 77], Russia [64], Iran [76, 77], Mongolia [101], China [101], Taiwan [101], USA [14], Canada [14], Afrika (Lybia, Egypt, Senegal)

[53]. *N. ericae ericae* is a subspecies of *N. ericae* which, due to the polymorphism characteristic of the species of the genus Nysius and the Lygaeids in general, is difficult to distinguish and determine, which explains the numerous synonymies and confusions over time. Moreover, quite a few authors confuse it with the type species or others consider it a useful species. We hope that further studies are necessary to establish his role in the biological control process of common ragweed. In our study, we documented the first recorded distribution of his species within the territory of Romania (Fig. 7).

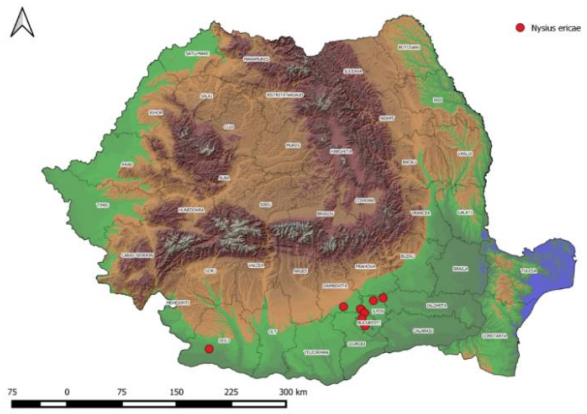


Fig. 7 – Map distribution of *Nysius ericae ericae* in Romania

Nysius cymoides (Spinola, 1837) (Heteroptera: Lygaeidae)

Synonyms: *Heterogaster exilis* Costa, 1855, *Macroparius cymoides* (Spinola, 1837), *N. fuliginosus* Fieber, 1861, *N. thoracicus* Horvath, 1882, *N. albidus* Dallas, 1852; *Artheneis cymoides* Spinola, 1837. Ithas been recorded in multiple regions, including Europe: Spain [93], Slovakia [90], Turkey [24,23,66,103], R.Moldova [29], France [69], Italy [16,65,50,27], Serbia [81], Iran [4,51,56,55,58], USA [85], Lybia [53], Sudan [53].

N. cymoides differs from other Euro-Mediterranean species for its modest size (3.5-4 mm) and the following characteristics: i) dorsal parts whitish-translucent colored; ii) general shape elongate and slender; iii) pronotum little, strongly transverse, its width is around two times the height; iv) membrane of the hemelytra hyaline and transparent, it surpasses the apex of the abdomen; v) buccula, in the lower part of the head, reduced progressively backward [16]. This species was also reported in Montenegro [81].

This species is highly polyphagous, with host plants documented in Turkey including *Abelmoschus esculentus*, *Capsicum annuum*, *Citrullus lanatus*, *Cucumis sativus*, *Cucurbita pepo*, *Helianthus annuus*, *Glycine max*, and many others [103].

In Italy, an outbreak of *N. cymoides* in soybean (*Glycine max*) fields in 2017 caused severe damage, with symptoms including desiccation, wilting, and necrosis of plant tissue, as well as seed weight loss [68,16]. Similar impacts may occur in other regions where the species is established [68,85,16,26].

In Romania, *N.cymoides* was previously listed in a compilation of species from the Danube Delta Biosphere Reserve (ARBDD, 2022), highlighting its presence in this ecologically significant area. Additionally, it was recently recorded on *A. artemisiifolia* (common ragweed) in other parts of the country. While other potential host plants were not assessed during this study, its current distribution in Romania is illustrated in (Fig. 8.)

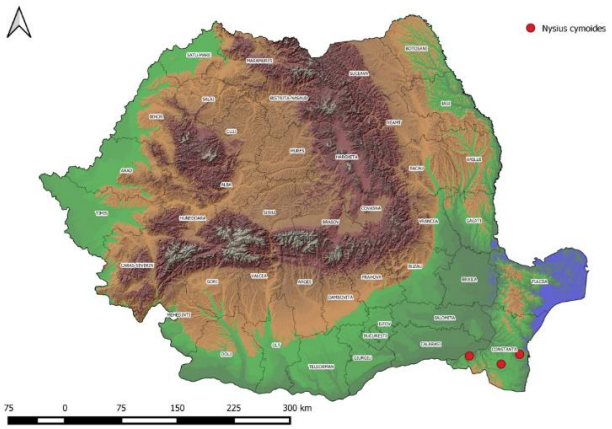


Fig. 8 – Map distribution of *Nysius cymoides* in Romania

Among the collected organisms, special attention was given to *Ophraella communa*, a leaf beetle with the potential for biological control of common ragweed.

Ophraella communa, LeSage, 1986 (Coleoptera: Chrysomelidae)

The ragweed leaf beetle, *O. communa* LeSage (Coleoptera: Chrysomelidae), is an oligophagous species indigenous to North America. Its primary food source is common ragweed, although it has also been observed on several other species within the Asteraceae family. In Australia, *O. communa* was deemed unsuitable for intentional

release after studies indicated its ability to complete its life cycle on sunflowers in controlled laboratory settings [67]. The beetle was later identified in Japan and Taiwan in 1996 [103], likely as a result of unintentional introductions, and was subsequently reported in South Korea [88] and China [54], with its first documentation in China occurring in 2001. Extensive laboratory and field studies, in addition to field surveys, have shown no significant non-target impacts of *O. communa* on sunflowers [113]. Since that time, the beetle has been mass-reared and released for the biological control of *A. artemisiifolia* across various regions [41, 113] and has established a widespread presence in China [113]. More recently, *O. communa* has been inadvertently introduced into Europe, with its initial detection occurring in Northern Italy, close to Milan airport [16], and subsequently in Southern Switzerland in 2013 [62]. In Northern Italy, the beetle is capable of producing as many as four generations annually [60]. The beetle has proliferated throughout Northern Italy [53] and has spread to Slovenia [90], Croatia in 2018 [105], Serbia [76], Hungary [46] and Bosnia and Herzegovina [45].In Romania was first detected near Bucharest in 2019 [47] and in Popești-Leordeni (Ilfov county) during 2020-2021, in the same year when we detected it for the first time in the area of Moneasa locality, Arad County. Complete defoliation was recorded at densities of 5-10 individuals/m². The distribution in Romania according to our study was presented in the map (Fig. 9).

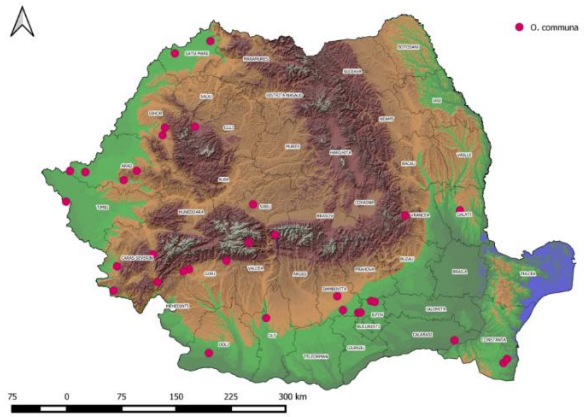


Fig. 9 – Distribution map of *Ophraella communa* in Romania

In Romania, *O. communa* is a multivoltine leaf beetle that overwinters at the adult stage and lays eggs in egg batches in spring. *O. communa* beetle was sometimes found on neighboring Asteraceae plants (mainly sunflowers). His behavior seems to be very similar to those of the Colorado beetle which it seems to be related. The beetle then goes through three larval stages, which feed on the green parts of the host plant. It then pupates and starts mating shortly after emergence from the lightly woven cocoon. Adults feed on green parts of the plant as well but often, mainly after the flouring phenophase of common ragweed (end month of August till September-October, depending on the weather conditions), the beetle also attacks even the stems. Economic assessments in Europe suggest that *O. communa* could substantially reduce the costs associated with managing *A. artemisiifolia* infestations. Studies in France [60] and other regions with suitable climates have highlighted its potential as a cost-effective biological control agent.

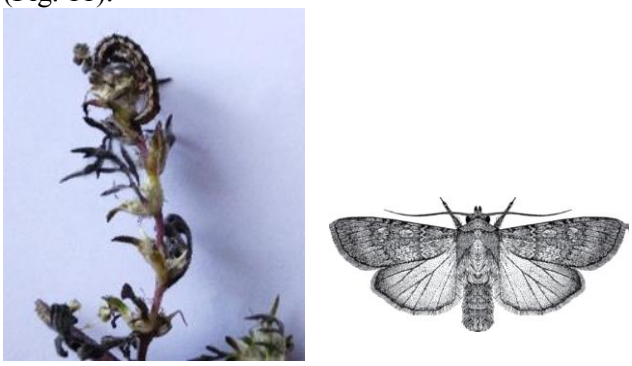
Peridroma saucia, (Hübner, 1808) (Lepidoptera:Noctuidae)

Variegated cutworm was found in many areas of the world. It occurs in North and South America, the Hawaiian Islands, and also portions of Europe, Asia, and North Africa. The origin of this insect is uncertain but is thought to be from Europe, where it was described in 1790. First observed in North America in 1841, it is now abundant in southern Canada and the northern United States, where was often considered to be the most damaging cutworm pest of vegetables. Other reports were from Argentina [94], Turkey [104], Brazil [87], Colombia [35] and China [102,41]. Although it is considered to be spread all over the globe, there is still little data on its spread in Europe or significant damage. From an agricultural point of view, it seems to be of minor importance: in Europe was reported from Finland [1].

There are 2–4 generations annually, with two generations common in colder regions such as Canada. The egg of variegated cutworm is hemispherical; the egg is flattened at the point of attachment to a leaf or plant stem. The surface of the egg is marked with ridges, about 42 in number, radiating from the center. The egg is 0.55–0.58 mm in diameter and 0.40–0.45 mm in height. Initially, eggs are white, but soon turn brownish. Females may deposit 1200–1400 eggs during their life span. The developmental threshold for the egg stage is estimated at 3.0–6.0°C. The duration of the egg stage is 4–6 days in warm weather (20–30°C), but 10 days when held at 15°C. Eggs are deposited in clusters, often numbering several hundred per egg mass [95]. There normally are six instars. The developmental threshold for the larval stage is estimated at 2.6–6.7°C [78, 79, 84]. Head capsule

widths are about 3.0–3.2 mm. The body color is brownish-gray to grayish-black. The most distinctive character is a dorsal yellow or whitish spot, present on each of the first 4 abdominal segments, often the first six segments, although this character may be absent in early instars. The variegated cutworm is described by many as one of the most damaging pests of vegetables. The moth causes damage by cutting off the plant at the surface of the soil, immediately destroying the crop. The larvae themselves are defoliators [18,19]. Some studies indicate the presence of species feeding on *A. artemisiifolia*, especially in autumn when other crops have finished their vegetation. *P. saucia* also feeds on weeds. Feeding on weeds is less common than feeding on fruits and vegetables, but has still been observed. The variegated cutworm feeds on the following weeds: *A. artemisiifolia*, *Datura stramonium* L., *Rumex crispus* L., *Eupatorium capillifolium* (Lam.) Small, *Capsella bursa-pastoris* Medik. Many of the larvae were collected alive and placed in special cages with a natural host plant to rear them under laboratory conditions and obtain adults (Fig. 10, a).

This study enabled the first identification of the alien species in 10 localities across Romania, observed in the larval stage feeding on *A. artemisiifolia* during its full flowering phenophase (Fig. 11).



a) b)

Fig. 10 - *Peridroma saucia* a) larvae, b) imago (courtesy of John L. Capinera)

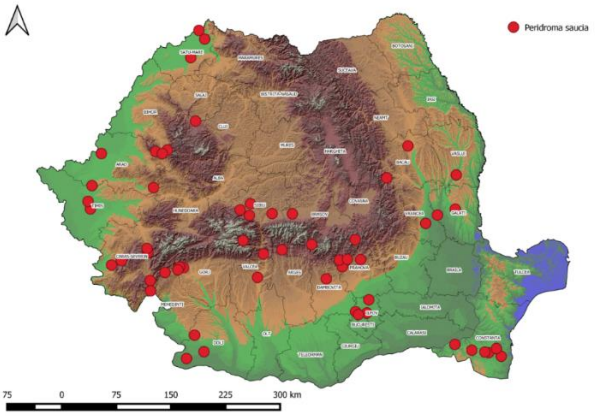


Fig. 11 – Map of *Peridroma saucia* distribution in Romania

Acontia candefacta (Hübner, 1831) (Lepidoptera: Noctuidae)

It is a Nearctic species originally described from Pennsylvania (USA). It is widely distributed across North America, from southern Canada to Mexico, but is more common in the southern half of its range. Biological control agent, *A. candefacta*, can be effectively used to control the widespread ragweed, *A. artemisiifolia*, as it consumes the leaves of the Ambrosia species (Fig. 12). This species has a complex life cycle, with two broods per year produced from May to September [3].

a) b)

Fig. 12 - *Acontia candefacta,* a) larvae, b) imago

In the 1960s, *A. candefacta* was purposefully imported to southern Russia, including the Krasnodar area, for biological control of *A. artemisiifolia* [72,73]. Several stable populations survived in Kuban for about 30 years, while the species did not spread. However, collections from Ukraine's Lugansk and Donetsk regions were made between 1999 and 2002, and it was also found in the

Rostov-on-Don region in the north in the same period [72]. Since then, the species has spread both east and west. Z. orientalis is now also established in the Kabardino-Balkar Republic (in northern Caucasus) [72] and very recently reported from Zaporozhye and Cherson (both in southeastern Ukraine).

Since its establishment, the expansion of the species has been slow but steady, with the species being recorded only in Serbia [96], Hungary [48,49], Croatia [49], Slovenia [49], and Bosnia and Herzegovina [49] after its arrival; more recently, in Poland [44]. Hence, *A. candefacta* is quickly spreading west across the steppes bordering the north of the Black Sea.

A. candefacta has been observed in Romania, and its continuing westward spread was recently predicted. The three males we obtained from northern Dobrogea and from Manolache (near Bucharest, 44°23′46″N, 26°14′30″E) are the first records for *A. candefacta* in Romania and bear witness to its westward extension. Soon after, its wing morphology allowed for the accurate identification of *A. candefacta*.

Within the Romanian fauna, *A. candefacta* is closely related to *Acontia lucida* (Hufnagel, 1766), *A. titania* (Esper, 1798), and *A. melanura* (Tauscher, 1809). However, it may be readily distinguished by its smaller size (wingspan of 19–23 mm) and its distinctive wing patterns, most noticeably the lack of an evident black submarginal band on the hindwings. This species is highly likely to be either present already or will spread into southern Romania, including southern Moldavia, Muntenia, and Oltenia [75]. The collection of *A. candefacta* in Bulgaria on the southern banks of the Danube (close to the Romanian border, south from the town of Corabia, S. Beshkov, pers. comm. to L. Székely 1994) [86]. This location is about 140 km further west than our farthest western record from Manolache. After its introduction into Romania and Bulgaria, the species seems to be advancing westward, typically following the course of the Danube River. However, recent records of a female caught at Balgarevo (Kaliakra, Bulgaria) suggest that *A. candefacta* are still spreading southwards along the Black Sea coastline. After a recent report suddenly from Poland, it is expected that this species will arrive in central Europe in a few years. The best results about the presence, distribution, and acclimatization of *A. candefacta* were obtained recently in the Republic of Moldova [91,92].

In Romania, the species had been well studied and documented in some relevant taxonomic papers [74]. *A. candefacta* attacks especially in the phenophase after flowering, in the middle of August, when it represents an attractive source of food for the species, because the vegetation is strongly affected by drought and high temperatures in the last months of summer. The leaves and inflorescence are consumed, which can represent an important factor in reducing the seed production of common ragweed. Actually, after our study, the distribution of species in Romania was presented on the map but this is only a dynamic situation that could be constantly changed (Fig. 13).

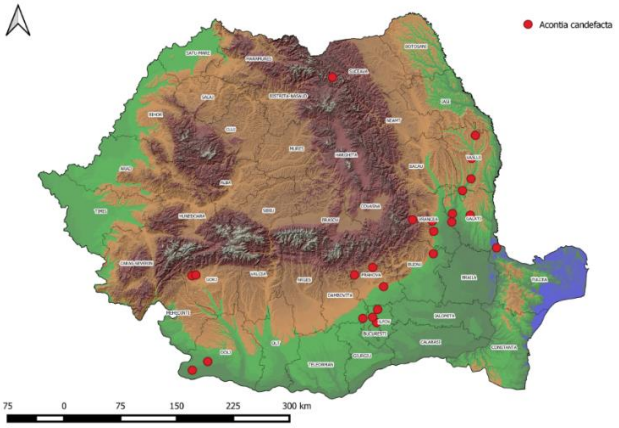


Fig. 13– Map distribution of *Acontia candefacta* (Hübner, 1831) in Romania

*Adaina ambrosiae (*Murtfeldt, 1880*)*

Lepidoptera: Pterophoridae

Synonims: *Pterophorus ambrosiae* Murtfeldt, 1880, *P. perplexus* Grossbeck, 1917, *A. ambrosiae*, Barnes & Lindsey, 1921. Originating in North America, from Central America to Toronto, Canada, in the Great Lakes region, little is known about this species, including its description and global distribution. Few papers have focused on its ecology and biology.

Recorded hostplants: *A. artemisiifolia* L., *A. acanthicarpa* Hooker, *A. chamissonis* (Lessing) Greene, *A. confertilora* Decandolle, *A. dumosa* (A. Gray) Payne, *A. eriocentra* (A.Gray) Payne, *A. cunanensis* HBK. (= *psilostachya* DC.), *Pluchea rosea* (Godfrey), *P. carolinensis* (Jacq.) G. Don., *Melanthera nivea* (L.) Small (= *M. deltoidea* Michx.), *Helianthus annus* L., *H. tuberosa* L., *Xanthium strumarium* L. (cocklebur), *Neurolaena lobata*, artichoke (leaves), *Rudbekia* sp. Some authors indicate that the food regime is similar like those of *O. communa* [38]. Except for the sunflower, his oligophagous trophic status indicates

that the favorite hosts are species of Ambrosia genus.

Our investigations show only the presence of this species in two localities from Romania on *A. artemisiifolia* on the west side of the country.

Regarding heteropteran fauna, notable research has been conducted in the Balkan region, particularly in Turkey. Two comparable studies to the present work were carried out between 2020 and 2022, with a literature review tracing back to 1922. These studies examined the host plant relationships of the superfamily Lygaeoidea in Turkey, discussing and summarizing the findings. The Lygaeoidea superfamily includes both phytophagous and zoophagous insects, displaying significant diversity in feeding habits. Most species feed on mature plant seeds, while others, such as many species of Lygaeidae, feed on plant sap [22,23]. Unfortunately, both studies referred only to the local or endemic Turkish flora and they did not consider invasive species like *A. artemisiifolia*. Another important study on European bug fauna was conducted in southern Slovakia between 2020 and 2021. This research focused on heteropteran species feeding on or inhabiting *A. artemisiifolia*. The most common phytophagous species observed were *N. ericae ericae* (Schilling, 1829) (Lygaeidae), *Adelphocoris lineolatus* (Goeze, 1778), *Lygus rugulipennis* (Poppius, 1911), and *Lygus pratensis* (Linnaeus, 1758) (Miridae). A zoophagous species, *Nabis* (Dolichonabis) *limbatus* (Dahlbom, 1851) (Nabidae), was also identified. Notably, *Halyomorpha halys* was the only detected carrier of phytoplasmas, though its abundance on *A. artemisiifolia* was extremely low [90], a finding consistent with our observations.

A similar study in Italy investigated Heteroptera species in dry grasslands within the Malpaga-Basella Nature Reserve. In the nearby *Miscanthus giganteus* crop, 51 heteropteran species from nine families were recorded. Among these, the family Lygaeidae comprised the most species, followed by Pentatomidae. Interestingly, in Italian Heteroptera, Miridae is the largest family, accounting for 34% of species, but in the Nature Reserve, it represented only 12% [50]. This contrasts with our study, where Miridae was the most abundant family, followed by Pentatomidae.

The Italian study also provided important data on the genus Nysius, reporting species such as: *N. cymoides* (Spinola 1837), *N. ericae* (Schilling 1829), *N. graminicola* (Kolenati 1845), *N. helveticus* (Herrich–Schäffer 1850), *N. senecionis* (Schilling 1829), species typical of dry grassland, four of them being present in our study. Another Italian study contributed a comprehensive checklist of the heteropteran fauna, documenting 1,395 taxa belonging to six infraorders and 48 families. This includes 44 endemic taxa, 12 sub-endemic, 13 introduced, and 10 cryptogenic species. The study also examined nearly 220 bibliographic sources from Italian scientific literature [27].

4 Conclusions

This study provides a detailed assessment of *Ambrosia artemisiifolia* in Romania, highlighting its distribution, natural enemies, and potential for biological control. The main findings include:

1. Identification of a significant number of natural enemies of *A. artemisiifolia*, which can be further studied and selected for biological control applications.
2. Development of a dynamic distribution map for *A. artemisiifolia* in 22 counties, covering 91 localities.
3. Collection of 4,550 arthropods using various methods, representing a diverse taxonomic structure that includes 9 insect orders and numerous arachnid species, particularly from the order Araneae.
4. Identification of the most abundant taxa, with Heteroptera being the dominant group (2,492 individuals, 103 genera, and 135 species from 18 families).
5. Discovery of 109 phytophagous species within Heteroptera, of which 15 are seed consumers, highlighting their potential as biological control agents.
6. Mapping the distribution of key species involved in *A. artemisiifolia* control, including *Ophraella communa*, *Acontia candefacta*, and *Peridroma saucia*.
7. Identification of five new species for the Romanian fauna: *Adaina ambrosiae*, *Nysius ericae ericae*, *Nysius huttoni, N. cymoides* (Spinola, 1837) and *N. helveticus* (Herrich-Schaeffer, 1850)

These findings significantly enhance our understanding of *A. artemisiifolia* and its associated arthropod fauna in Romania. The results serve as a foundation for future research on biological control methods and underline the importance of integrating local and alien species into management strategies for invasive species.

Acknowledgments

Author contributions
Dr. Traian Manole conceived the study, collected field samples, conducted the laboratory sample processing and wrote the draft.
Dr. Ana Cristina Fătu was responsible for laboratory sample processing, refining specific passages of the text, and formatting the manuscript.
Dr. Viorel Fătu analyzed the data and made the maps.

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