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Mochary example: how the highest European local species richness of pimpliform ichneumonid parasitoids (Ichneumonidae: Pimplinae, Poemeniinae and Rhyssinae) is under threat of disappearance in the Carpathian forests

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Abstract

A small forest patch (approx. 250 ha) of the Ukrainian Carpathian forest revealed an unexpected diversity of ichneumonid parasitoids: 95 species of Pimplinae, Poemeniinae and Rhyssinae), comprising appoximately 39 % of the total number of European species. This is the highest local species richness of the group ever recorded in Europe and one of the highest in the World. These results were obtained due to the use of a long-term multi-method sampling programme. Being extremely diverse and highly specialized ichneumonids (saproxylic parasitoids especially) are relatively vulnerable to habitat destruction and endangered because of deforestation. This is important for conservation of natural Carpathian forests and understanding the requirements for further study and estimation of the studied group species richness.

Key words: Hymenoptera, Ichneumonidae, Pimplinae, Rhyssinae, Poemeniinae, parasitoids, species richness, conservation, Ukraine

Introduction

Illegal logging is a major environmental and economic problem, and exceeds in some countries the amounts of legally harvested timber. Illegal logging appears to have been at least as extensive as documented logging during the early 1990s and the so-called 'sanitary clear-cuts' represent a major loophole for overharvesting and logging in restricted areas, especially across the Ukrainian Carpathians (Cuemmerle *et al*. 2009). Compared to other biota-rich European mountain systems, the Carpathians' geographical position, isolation, area, landscape heterogeneity, and relatively well-preserved environment, as well as the lower impact of Quaternary glaciations, have helped to buffer against the negative effects on biological richness caused by the overall lower altitude of the massif and the absence of adjoining Mediterranean biodiversity hotspots. In particular, the Carpathians include one of the largest continuous forests in Europe and harbour some of the last remaining stands of primeval forest. The Carpathian forests not only have some of the largest populations of large mammals in Europe, but are an important diversity hotspot for many groups of invertebrates, including insects (Mráz & Ronikier 2016).

Hexapoda is one of the most species-rich groups of organisms, including those with a parasitoid lifestyle, including hymenopterans of the family Ichneumonidae (*e.g.* Townes 1969). Parasitoids are insects whose larvae develop by feeding in or on other arthropods, often insects, which results in the death of the parasitoid's host (Godfray 1994). Parasitoids are often specialized and occupy high trophic levels, which makes parasitoid populations dependent on the abundance and distribution of suitable hosts and thus particularly vulnerable to different levels of extinctions if host availability drops (Shaw & Hochberg 2001). Furthermore, habitat fragmentation affects parasitoids more than their hosts, suggesting that specialized parasitoids might be more sensitive to habitat change (Komonen *et al*. 2000). The Carpathian forest biodiversity is disappearing at an alarming rate owing to human activities, accentuating the need for accurate assessment of parasitoid diversity (Hostert 2009). Shaw and Hochberg (2001) even discuss the utility of conservation initiatives that do not take parasitoids into account, largely because of our ignorance of them, both taxonomically and biologically. The subfamily Pimplinae is taxonomically and biologically one of the best-known ichneumonid subfamilies (Gauld *et al.* 2002). Thus, pimplines (together with some other Ichneumonidae subfamilies)

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have been used in studies of latitudinal trends of ichneumonid parasitoid species richness and have been suggested to be less species rich in the tropics than in temperate zones (*e.g.* Owen & Owen 1974). In contrast, high tropical species richness of the group has been discovered in tropical areas of Mesoamerica recently (Saääksjärvi *et al*. 2004; Gómez *et al.* 2017). However, it is still a challenge to answer a question: what do we actually know about temperate parasitoid species richness, even in such a relatively well-studied area as Europe?

The revisionary works of Aubert (1969), Fitton *et al*. (1998), Kasparyan (1981) and others made Pimplinae wasps rather well-known in Europe. The Carpathian Basin was also intensively investigated: Bajári (1960) studied ichneumonids of Hungary, Sedivy (1989) provided a list of ichneumonids of the former Czechoslovakia, Constantineanu and Pisica (1977) studied Romanian parasitoids. Despite that, the Ukrainian Carpathians remained a 'white spot', as most of the Ukrainian data provided by Kasparyan (1981) covered the Eastern and Southern parts of Ukraine. During the last few years the Ukrainian Carpathian ichneumonid fauna, especially the subfamily Pimplinae, has been intensively sampled by the author (Varga 2012, 2014d, 2017ab, 2018a, 2018b). From these studies it has become clear that ichneumonids are diverse and relatively unknown in Carpathian forests. The main goal of the present study was to shed light on local species richness of Pimplinae, Poemeniinae and Rhyssinae based on a long-term multi-method sampling programme established by the author in three small Carpathian forest sites.

Material and methods

The study area

The present study was conducted in three closely situated localities (the 'Mochary' forest (48.838638 N, 24.579456 E), 55 ha, the 'Dibrova' forest (48.769889 N, 24.504539 E), 27 ha, and the 'Zhbyr' forest (48.771726 N, 24.453764 E), 175 ha), which are located 5–8 km around the town of Bogorodchany (Ivano-Frankivsk Region) in the Precarpathian lowland (Fig. 1). The study area belongs to the Carpathian Montane Forests ecoregion (Olson *et al.* 2001). The studied localities lie between 310 and 340 m above sea level and are characterized by the mainly turf-podzolic soils, moderate continental climate with a mean annual precipitation between 630 and 690 mm, an average temperature of -4.8˚C in January and +17.9˚C in July, and a vegetation period of 190–215 days per year (Gerenchuk 1968). According to Shelyag-Sosonko *et al.* (1991) the vegetation of the studied localities are broadleaf forests with *Caprineto (betulae) - Quercetum (roboris) aegopodiosum (podagrariae)* and *Caprineto (betulae) - Quercetum (roboris) vincorum (minoris)* plant associations.

Specimen collecting methods

The present study is based on 1752 specimens collected by the author between 2009 and 2018, of which only 1561 specimens were used in the study (for details see 'Species identification' section). Several types of traps were used: Malaise, Trunk (so-called 'Tereshkin') and yellow pan traps. The Malaise trap (Figs 2–3) is a tent-like structure that has been widely used for collecting major insect groups, including ichneumonids, in similar studies (*e.g.* Saääksjärvi *et al*. 2004; Gómez *et al.* 2017). The Trunk trap is a conical structure (Fig. 6) that is not commonly used in studies on parasitoids, but this method was found to be relatively efficient in collecting saproxylic parasitoids (Varga 2014abcd, 2015ab, 2017c). The yellow pan traps were not productive, except those that were placed on or near dead trunks (Fig. 5). The two Malaise traps were placed in the Mochary locality (Fig. 1) in 2014, 2015 and one trap in 2018 in different parts of the forest, e.g. inside the forest, near a dead *Picea abies* trunk or on the borderline between the forest and agricultural land. The total sample size for all 5 traps was 617 Malaise trap days or 20.6 Malaise trap months (MTM). One Trunk trap was placed in Mochary on a dead *Picea abies* trunk in 2014 for 135 trap days (Fig. 1). The Trunk trap in the Zhbyr locality placed in 2013 was almost ineffective due to a *Vespa crabro* nest being constructed inside at the beginning of the sampling period. Yellow pan trap were placed several times for periods of 2–3 days in the Zhbyr locality. Sampling by traps can often be prevented by several factors (experience suggests) (Fig. 2):

- traps can be partly destroyed by strong wind, icefall, falling branches, large mammals or people;
- the uppermost point of a trap (a hole connected to the gathering chamber) can be blocked by netting spiders, nesting wasps or small mammals, e.g. dormice (Fig. 3);
- gathering chambers can be thrown over by people despite warning signs.

Also, additional sampling methods, such as sweep netting and rearing from hosts, were used in the study. Two types of nets were used: large (with a larger diameter approx. 60 cm), used for sweeping branches and shrubs, and smaller (with a diameter approx. 35 cm) used for selective collecting of saproxylic parasitoids around dead trunks (Fig.

4). Rearing from hosts was used for the species living in an environment hardly accessible by netting, e.g. logs, cones or acorns (confined to canopy), or the spider associates. All these approaches allowed an estimate as close as possible to the real local species richness of the group.

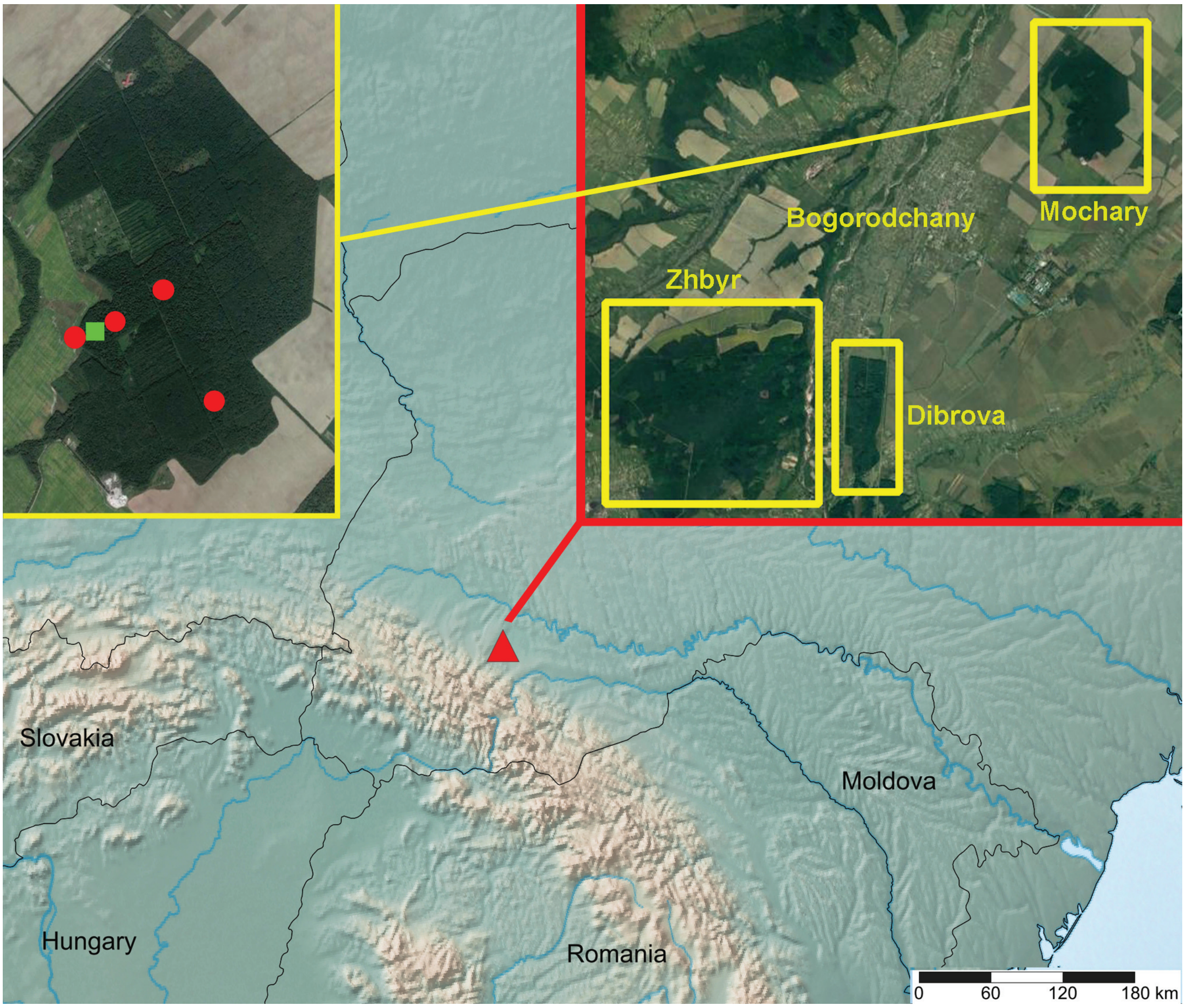


Figure 1. Location of the study area in the Precarpathian lowland. Red circles indicate Malaise trapping site. Green squares indicate Trunk trapping sites.

Taxonomic efforts and correct species identification

All the collected pimpliformes were sorted to genera level. Each genus was sorted into morphospecies and identified to species level, except some *Endromopoda* and *Scambus* specimens. Whenever possible, males were sorted only after females found in the sample. Records based only on males in 'taxonomically difficult' genera (*Endromopoda*, *Scambus*) were not included in the analysis. When taxonomic placement of a given taxon was challenging, comparative material (types and non-types) from museum collections were used for resolving such situations. The materials collected in the studied area and the comparative materials were analyzed and partly listed in the revisions of particular ichneumonid subfamilies/tribes, previously published by the author (Varga 2012, 2014d, 2017ab, 2018a, 2018b). The voucher specimens from this study are mainly deposited at Schmalhausen Institute of Zoology, NAS of Ukraine, Kyiv (SIZK). Each species is represented by several pinned specimens placed in the main collection boxes. The rest of the specimens (about 60 % of material) are stored in 96 % ethanol.

To describe assemblage structure, the species occurring in the study area were divided into four categories according to Gauld (1991):

1. Idiobiont parasitoids of deeply concealed hosts (species with very long ovipositors are classified here, including all rhyssines and poemeniines and several pimpline genera, namely *Atractogaster*, *Perithous*, *Pseudorhyssa*, *Dolichomitus*, *Ephialtes*, and *Townesia*).

Figures 2–6. Collecting methods in the study area. **2–3,** Malaise trap; **2,** General view of the trap; **3,** Damaged trap. Red arrow indicates the most vulnerable part of the trap; **4,** Selective collecting of saproxylic parasitoids near dead trunk by net; **5,** Yellow pan traps; **6,** Trunk trap.

2. Idiobiont parasitoids of rather weakly concealed host species (most of the Pimplini species, *Delomerista* and a vast majority of ephialtines).

3. Pseudoparasitoids feeding in egg sacs, or on spiders guarding sacs (only the species of Ephialtini genera *Clistopyga*, *Tromatobia* and *Zaglyptus*).

4. Koinobiont parasitoids of adult spiders (represented by the following genera: *Acrodactyla*, *Oxyrrhexis*, *Polysphincta*, *Schizopyga*, and *Zatypota*).

Results and discussion

In total, 95 Poemeniinae, Pimplinae and Rhyssinae species were collected in the study area. Among 83 collected pimpline parasitoid species there are nine delomeristines, 58 ephialtines, 15 species of the tribe Pimplini and one of Theroniini.

Most of the delomeristines are commonly flying around dead branches, but at the same time are mostly undersampled by traps placed near these trees. The Pimplini species, as well as many ephialtines, were sampled successfully by both trapping and netting. However, two Ephialtini species, *Clistopyga incitator* (Fabricius, 1793) and *Oxyrrhexis carbonator* (Gravenhorst, 1807), and one Pimplini species, *Itoplectis melanocephala* (Gravenhorst, 1829), were collected only by traps, whereas another two ephialtines, *Scambus calobatus* (Gravenhorst 1829) and *Liotryphon strobilellae* (Linnaeus, 1758), were reared only from acorns and cones, respectively. The Mochary locality houses several small patches with dead conifers. However, 17 *Dolichomitus* species (among 25 European species) were surprisingly collected here. Most of the idiobiont parasitoids of deeply concealed host species were largely unsampled by Malaise traps, specimens of these were numerous in the Trunk traps and were also easily collected by selective sampling by a small net near dead tree trunks. The subfamily Rhyssinae was represented by all of the European species of the genera *Rhyssa* Gravenhorst, 1829 and *Rhyssella* Rohwer, 1920, and by only a single species of the genus *Megarhyssa, M. rixator* (Schellenberg, 1802), associated with conifers in the studied area. The poemeniines collected in the studied area belong to the genera *Deuteroxorides* (1 species), *Neoxorides* (2 species) and *Poemenia* (all four European species).

The assemblage structure of Pimplinae s.l. fauna, together with a comparison of some other well-studied localities, is presented in Table 1. In general, the biological composition of pimplines and rhyssines in the study area of the Precarpathian forests is rather similar to other known European study localities. Generally, idiobiont parasitoids of weakly concealed pupae or cocoons predominate (37.4 %) among other pimpliform groups in the studied area. However, ectoparasitoids of spiders comprise only 9.8 % of species of the target group.

Table 1. The biological composition of the Pimplinae and Rhyssinae in four temperate and one tropical sites (modified after Gauld, 1991; Saääksjärvi *et al*. 2004). Numbers in parentheses indicate percentages of the total species number at sites. Original data: Finland (Jussila 1984), England (Owen *et al*. 1981), Poland (Sawoniewicz 1986), Peru (Gómez *et al.* 2017), Ukraine (Varga 2012, 2014cd, 2015a, 2017ab, 2018a, 2018b, Varga, in prep.).

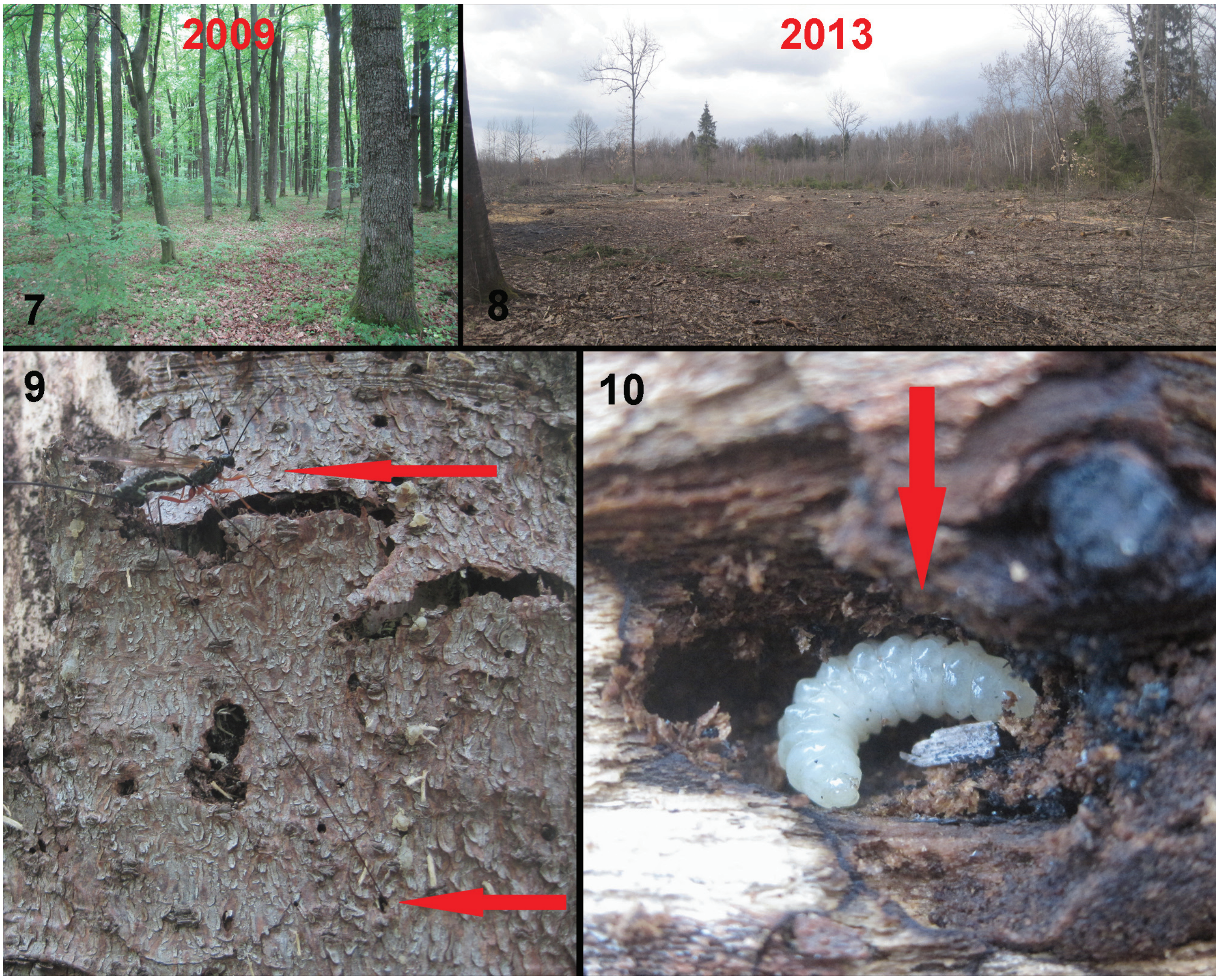
	Finland Kevo	England Leics	Poland Warsaw	Peru Allpahuayo-Mishana	Ukraine Precarpathia
Idiobiont parasitoids of deeply concealed hosts	7 (36.8)	4 (22.2)	11 (39.3)	18 (17.2)	34 (37.4)
Idiobiont parasitoids of weakly concealed pupae or cocoons	7 (36.8)	5 (27.8)	10 (35.7)	55 (52.4)	41 (45.1)
Idiobiont ectoparasitoids of spiders/ seudoparasitoids of spider egg sacs	1 (5.3)	3 (16.7)	2 (7.1)	3 (2.8)	7 (7.7)
Koinobiont ectoparasitoids of spiders	4 (21.1)	6 (33.3)	5 (17.9)	29 (27.6)	9 (9.8)
Total pimpliform species at site	19	18	28	105	91

Thus, 95 Poemeniinae, Pimplinae and Rhyssinae species collected in the studied area in Precarpathia demonstrate the highest documented local species richness of the group in Europe and at least one of the highest in the World.

The Mochary locality shows the highest local species richness if compared to other European localities, by comparing data from Malaise traps (Table 2). Furthermore, the non-European localities with similar sampling effort (around 20 MTM) also usually show lower values of species collected. In the most species-rich Peruvian site, Allpahuayo-Mishana, the Malaise trap proved to be the most efficient collecting technique, as all the Pimplinae and

Rhyssinae species encountered in the inventory were found in Malaise traps (Gómez *et al.* 2017). In contrast, I found Malaise trapping to be a good technique, but only as part of a multi-method sampling: only half of the species recorded in the studied area were collected by Malaise traps.

Unfortunately, the unique insect community of the Mochary forest is endangered and may disappear in the very near future. Ichneumonid parasitoids, chiefly pimplines, being extremely biologically diverse (*e.g.* Gauld *et al.* 2002) and often highly specialized (occupy high trophic levels) are quite susceptible to environmental disturbances (Gibb and Hochuli 2002). The parasitoids living in dead wood, namely saproxylic parasitoids (Figs 9–10), are especially vulnerable. Komonen *et al*. (2000) found that habitat fragmentation affects saproxylic parasitoids more than their hosts, suggesting that parasitoids might be more sensitive to habitat change. It is no wonder that pimpliform species belonging to this biological group were included in the Red Databook of Ukraine. The Mochary locality is a habitat of the protected species *Dolichomitus cephalotes* (Holmgren, 1860) (Fig. 9). Furthermore, two additional species, *Megarhyssa rixator* and *Rhyssa kriechbaumeri* Ozols, 1973, were proposed to be included in the next edition of the Red Databook (Varga 2018c). Both species are widely distributed in the Ukrainian Carpathians and were found in Mochary. Additionally, this forest is the type locality of a recently described species, *Dolichomitus sirenkoi* Varga, 2012. Unfortunately, no specimens of this species have been collected since 2013, mostly because of habitat destruction by illegal logging (Figs 7–8). As is the case with tropical forests (Solar *et al*. 2015), Mochary is a local example of a global problem of biodiversity extinction, yet before that biodiversity is discovered. The creation of forest 'islands' on clear-cuts would improve habitat availability for saproxylic parasitoids for a period. But a diversity of dead wood substrates is required to support the diverse community of forest parasitoids, even when their regular host is present (Hilszczajski *et al.* 2005). Thus, the creation of an entomological reserve is recommended to save such a unique locality as Mochary before it is fully destroyed by humans.



Figures 7–10. The Mochary locality. **7–8,** Habitat destruction; **9,** The Red-listed *Dolichomitus cephalotes*, ovipositing into a *Picea abies* trunk. Red arrows indicate localization of specimen and oviposition hole; **10,** *Dolichomitus* sp. larva inside trunk.

Nowadays, data on biodiversity are frequently collected mostly in tropical areas where biodiversity is thought to be high (*e.g.* Saääksjärvi *et al*. 2004; Gómez *et al.* 2017), leaving the biodiversity of other areas largely neglected. Surprisingly, the diversity of invertebrates may appear concealed if a limited number of collecting approaches is used. The diverse research approaches and rigorous persistent research of the same plot over the years, are expected to reveal such 'concealed' diversity in various areas of the Earth.

Table 2. Species-richness of Pimplinae and Rhyssinae in 10 sites sampled with Malaise traps around the World. Modified after Gómez *et al.* (2017). Red – the highest recorded species richness. Blue – non-European localities with sampling effort around 20 MTM. Yellow – European localities. MTM: Malaise trap months.

Country	Locality	Species	Individuals	MTM	Reference
Peru	Allpahuayo-Mishana	105	1597	240	Gómez *et al.* 2017
Costa Rica	San Vito	28	88	18	Gaston & Gauld 1993
Iran	Orkom	12	64	16.6	Mohammadi *et al.* 2013
Malaysia	Sungkai	8	22	21	Idris & Kee 2002
Ukraine	**Mochary**	**44**	**113**	**20.6**	**In this study**
UK	York	41	1836	60	Mayhew *et al.* 2009
Spain	El Ventorrillo	33	428	12.6	Nieves-Aldrey *et al.* 2003
France	Bezange à bois mort	32	2412	36	Robert 2011
Italy	Bosco della Fontana	25	3945	56	Di Giovanni *et al.* 2015
Greenland	Northeast National Park	1	-	21	Várkonyi & Roslin 2013

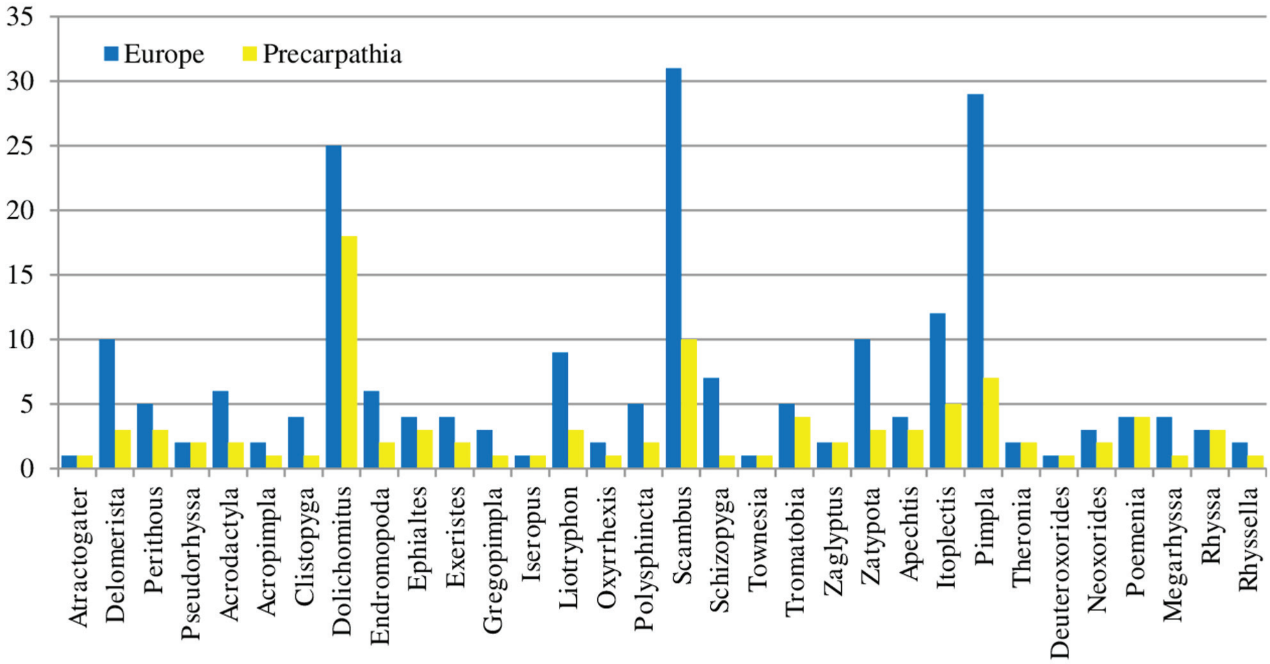


Figure 11. Number of species/morphospecies collected in the study area as compared to described pimpliformes in Europe (after Yu *et al*. 2012; Varga 2012, 2014cd, 2015a, 2017ab, 2018a, 2018b, Varga, in prep.).

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