

PAUESIA QUILIS, 1931 (HYMENOPTERA, BRACONIDAE; APHIDIINAE) IN SERBIA: THREE SPECIES NEW TO SERBIAN FAUNA WITH AN IDENTIFICATION KEY

KORANA KOCIĆ^{1*}, JELISAVETA ČKRKIĆ^{1,2}, ANDJELJKO PETROVIĆ¹, and ŽELJKO TOMANOVIĆ^{1,3}

1 University of Belgrade, Faculty of Biology, Institute of Zoology, Studentski trg 16, Belgrade, Serbia
*E-mail: korana.kocic@bio.bg.ac.rs (corresponding author), jckrkic@bio.bg.ac.rs, andjeljko@bio.bg.ac.rs,
ztoman@bio.bg.ac.rs

2 Centre for Biodiversity Genomics, University of Guelph, 50 Stone Road East, N1G 2W1, Guelph, Ontario, Canada
3 Serbian Academy of Sciences and Arts, Knez Mihailova 35, 11000, Belgrade, Serbia

Abstract

Pauesia is a diverse genus within the Aphidiinae subfamily, comprising about 80 species across the Holarctic region. Despite their diversity and distribution, a comprehensive modern revision of the species has yet to be undertaken. So far, six species have been recorded in Serbia. Our study identified three species new to the Serbian fauna: *Pauesia similis* Stary, 1966, *P. juniperorum* (Stary, 1960), and *P. silvestris* (Stary, 1960). Data on the location and tritrophic associations of the collected material are provided, as well as an identification key for *Pauesia* females in Serbia. We present two new trophic associations, both involving *Pinus peuce* Griseb. (Macedonian pine): *P. similis* and *P. piceaecollis* reared from *Cinara* aphids.

KEY WORDS: Serbia, aphid parasitoids, species, identification key

Introduction

Pauesia is a species-rich genus within the Aphidiinae subfamily, consisting of around 80 species distributed across the Holarctic region (Yu *et al.*, 2016). Nearly half of the species (33) have been described in Europe over the past century.

Based on the shape of the ovipositor sheath, species are classified into three subgenera: *Paraphidius* Stary, *Pauesia* Quilis s. str. and *Pauesiella* Sedlag and Stary (Davidian, 2015). With the few exceptions (such as *Pauesia antennata* Mukerji, 1950 that parasitizes *Pterochloroides persicae* Choldkovsky on *Prunus* sp.),

species of this genus are specialized parasitoids of *Cinara* aphids found on conifers, making them potential biocontrol agents (Kfir *et al.*, 1985; Kamanga-Thole *et al.*, 2021; Sopow *et al.*, 2021). However, despite its high species number and wide distribution, a modern comprehensive revision of genus *Pauesia* has not yet been conducted. This can be attributed to several factors, including high intraspecific variation in key morphological traits, subtle interspecific morphological variations among multiple species, and the absence of updated literature or revised species descriptions (personal observation).

In Serbia, no studies have focused exclusively on the genus *Pauesia*. To date, six species have been recorded. Kavallieratos *et al.* (2016) reported *Pauesia cupressobii* (Starý, 1960), *P. picta* (Haliday, 1834), *P. pini* (Haliday, 1834) and *P. unilachni* (Gahan, 1927), while Tomanović *et al.* (2021) documented *P. anatolica* Michelena, Assael & Mendel, 2005 and *P. piceaecollis* (Starý, 1960). In this study, we present three additional *Pauesia* species new to the Serbian fauna: *P. similis* Starý, 1966, *P. juniperorum* (Starý, 1960) and *P. silvestris* (Starý, 1960). We also provide an identification key for *Pauesia* females found in Serbia (Table I).

Material and Methods

Specimens were collected during the period from 2013 to 2019, primarily from high mountain areas in Serbia. All samples were reared from aphid colonies collected from conifer plants or as individual aphid mummies found on plant needles. Live aphids were transferred to 70% ethanol and later identified to the species or genus level. The samples were kept in plastic boxes with muslin covers for ventilation under laboratory conditions (22-24°C, 18:6 LD photoperiod) until the parasitoids emerged. Once eclosed, the specimens were transferred to 96% ethanol. Initial examinations were conducted using a ZEISS Discovery V8 stereomicroscope (Carl Zeiss MicroImaging GmbH, Göttingen, Germany). The specimens were then dissected and slide-mounted in Berlese medium. Photographs of the dissected specimens were taken using a Leica DM LS phase contrast microscope (Leica Microsystems GmbH, Wetzlar, Germany). The images were stacked using Helicon Focus software (version 7.6.1; www.heliconsoft.com) and all the important taxonomic characters (malar index, longitudinal eye diameter, tentoriocular line, intertentorial line, length and width of the first and second flagellomere, length and width of pterostigma, length of R1 vein, length and width of tergite I (petiole), length and width of ovipositor sheath) were measured using ImageJ software (Schneider *et al.* 2012). Other descriptive characters included the number of antennal segments, shape of the dorsal surface of the propodeum, shape of tergite I and ovipositor sheath, and specimen coloration. Morphological terminology follows Sharkey and Wharton (1997). The species records before 2013 can be found in Kavallieratos *et al.* (2004; 2016). The list of species documented in this study was primarily based on sampling focused on identifying parasitoids of aphids that are exclusively found on conifer plants.

Results

List of tritrophic associations

*species new to Serbian fauna

Pauesia anatolica Michelena, Assael & Mendel, 2005

Material: *Cinara cedri* Mimeur on *Cedrus* sp., 10 ♀♀, 8 ♂♂, Niš, 27.09.2019, leg. OPO.

Pauesia cupressobii (Starý, 1960)

Paraphidius cupressobii Starý, 1960

Material: **Cinara juniperi** (De Geer) on *Juniperus communis* L.: 4♀♀, 4♂♂, Bosilegrad, Jarešnik, alt. 1,230 m, 22.07.2013, leg. AP; 4♀♀, 2♂♂, Golija, Joše, alt. 939 m., 24.07.2016, leg. KK; 1♀, 1♂, Tutin, Draga, alt. 1,183 m., 04.07.2018, leg. JČ; 4♀♀, 3♂♂, Golija, put Odračnica–Raška, alt. 1,692 m., 05.07.2018, leg. KK; 3♀♀, 1♂, Golija, Lijeva Reka, alt. 988 m., 07.07.2018, leg. JČ; 4♀♀, 1♂, Golija, put Odračnica–Raška, alt. 1692 m., 08.07.2018, leg. KK; 1♀, Kopaonik, Rudnica, alt. 571 m., 10.07.2018, leg. JČ; 2♀♀, Kopaonik, Samokovska Reka, alt. 1,510 m., 10.07.2018, leg. KK; 4♂♂, Kopaonik, put Konaci–Kriva reka, alt. 1,401 m., 11.07.2018, leg. JČ; 1♀, 1♂, Tara, Zaovinsko Jezero, alt. 914 m., 18.07.2018, leg. KK; 1♂, Tara, Lazići, alt. 974 m., 19.07.2018, leg. JČ; 1♀, Zlatar, Aljinovići, alt. 1,199 m., 22.07.2018, leg. KK; 2♀♀, Zlatar, Vodene Poljane, alt. 1,463 m., 22.07.2018, leg. JČ; 8♀♀, 1♂, Stara Planina, Midžor, alt. 1,583 m., 01.08.2018, leg. JČ; 4♀♀, 9♂♂, Stara Planina, Babin Zub, alt. 1,327 m., 01.08.2018, leg. KK.

Pauesia juniperorum (Starý, 1960) *

Paraphidius juniperorum Starý, 1960

Material: **Cinara juniperi** on *Juniperus communis*: 4♀♀, 1♂, Golija, Muhovo, alt. 960 m., 25.07.2016, leg. JČ; 1♀, 1♂, Tutin, Ponor, alt. 1,738 m., 06.07.2018, leg. KK; 2♀♀, Kopaonik, Markov Kamen, alt. 1,684 m., 12.07.2018, leg. JČ.

Pauesia piceaecollis (Starý, 1960)

Material: **Cinara brauni** Börner on *Pinus* sp.: 2♂♂, Kopaonik, Mure, alt. 806 m., 10.07.2018, leg. KK; **Cinara pilicornis** (Hartig) on *Picea abies*: 6♀♀, 6♂♂, Stara Planina, Midžor, alt. 1,583 m., 01.08.2018, leg. KK; **Cinara** sp. on *Picea abies*: 4♀♀, Tutin, Ponor, alt. 1,738 m., 06.07.2018, leg. JČ; 2♀♀, Tutin, Ponor, alt. 1,738 m., 06.07.2018, leg. JČ; on *Pinus nigra*: 5♂♂, Kopaonik, Mure, alt. 806 m., 10.07.2018, leg. KK; 1♀, 1♂, Zlatar, Golo Brdo, alt. 1,596 m., 22.07.2018, leg. JČ; 3♀♀, 1♂, Zlatar, Vodene Poljane, alt. 1,463 m., 22.07.2018, leg. KK; on *Pinus peuce*: 1♀, Tutin, Pogled, alt. 2,154 m., 05.07.2018, leg. JČ.

Pauesia picta (Haliday, 1834)

Ichneumon aphidum (Curtis, 1837)

Aphidius varius (Curtis, 1837)

Aphidius varius (Smith, 1853)

Aphidius varius (Marshall, 1900)

Aphidius varius (Gaulle, 1908)

Material: **Cinara costata** (Zetterstedt) on *Picea abies*: 1♂, Golija, Jankov Kamen, alt. 1,833 m., 07.07.2018, leg. KK; 1♀, Golija, put Odračnica–Raška, alt. 1,567 m., 08.07.2018, leg. JČ; 1♀, Kopaonik, put Konaci–Kriva Reka, alt. 1,440 m., 11.07.2018, leg. JČ; 2♂♂, Kopaonik, Kriva Reka, alt. 1,161 m., 12.07.2018, leg. KK; 3♀♀, 3♂♂, Tara, Zaovinsko Jezero, alt. 914 m., 18.07.2018, leg. JČ; **Cinara** sp. on *Picea abies*: 1♀, 1♂, Stara Planina, Midžor, alt. 1,583 m., 01.08.2018, leg. KK; 3♀♀, Stara Planina, Midžor, alt. 1,583 m., 01.08.2018, leg. JČ; 1♀, 2♂♂, Stara Planina, Put za tri čuke, alt. 1,681 m., 03.08.2018, leg. KK.

Pauesia pini (Haliday, 1834)

Pauesia (*Paraphidius*) *planistipes* (Nees, 1834)

Pauesia (*Paraphidius*) *varia* (Nees, 1834)

Pauesia (*Paraphidius*) *panzerii* (Rondani, 1848)

Pauesia (*Paraphidius*) *lachnivorus* (Ashmead, 1906)

Material: **Cinara** sp. on *Pinus sylvestris*: 1♀, Golija, Jankov Kamen, alt. 1,833 m., 08.07.2018, leg. JČ.

Pauesia silvestris* (Stary, 1960) *Paraphidius silvestris* Stary, 1960

Material: *Cinara* sp. on *Pinus sylvestris*: 1♀, 1♂, Golija, put Odvracenicica–Novi Pazar, alt. 1,699 m., 08.07.2018, leg. KK.

***Pauesia similis* Stary, 1966 ***

Material: *Cinara* sp. on *Picea abies*: 1♀, Kopaonik, put Konaci–Kriva Reka, alt. 1,440 m., 10.07.2018, leg. JČ; 1♀, Stara Planina, Arbinje, alt. 1,364 m., 02.08.2018, leg. KK; on *Pinus peuce*, 18♀♀, 9♂♂, Tutin, Pogled, alt. 2,154 m., 05.07.2018, leg. JČ.

Pauesia unilachni* (Gahan, 1927)Pauesia albuferensis* Quilis, 1931*Pauesia praevisus* (Gautier & Bonnamour, 1936)*Pauesia basilewskyi* (Benoit, 1955)

Material: *Cinara* sp. on *Picea abies*: 1♀, Stara Planina, Arbinje, alt. 1,364 m., 02.08.2018, leg. KK; on *Pinus sylvestris*: 1♀, 1♂, Zlatar, Vodene Poljane, alt. 1,463 m., 22.07.2018, leg. JČ.

Abbreviated names of the sample collectors: Jelisaveta Črkić (JČ), Olivera Petrović Obradović (OPO), Korana Kocić (KK), Andjeljko Petrović (AP).

Table I. Key to the identification of female *Pauesia* species in Serbia

1.	Ovipositor sheath wide and stout (Fig. 1a)	2.
-	Ovipositor sheath of other shapes (Fig. 1b–1d)	7.
2.	Antennae with 23 antennomeres, pterostigma 2.3–2.4 times as long as wide.	<i>P. pini</i> Haliday
-	Antennae with 18–21 antennomeres, pterostigma more elongated, 2.6–3.1 times as long as wide.	3.
3.	Pterostigma 2.9–3.1 times as long as wide, tergite I stouter, 2.6–3.1 times as long as wide at spiracle level.	4.
-	Pterostigma 2.5–2.8 times as long as wide, tergite I more slender, 3.1–3.6 times as long as wide at spiracle level.	5.
4.	Body brown, mesoscutum brown or brown with yellow lateral lobes, tergite I rugose at the basal half (Fig. 2a), antennae with 19–20 antennomeres, parasitoid of <i>Cinara</i> aphids on <i>Pinus</i> and <i>Picea</i> .	<i>P. piceaeollis</i> Stary
-	Body generally light colored, mesoscutum yellow rufous to completely yellow, tergite I smooth at the basal half (Fig. 2b), antennae with 18–19 antennomeres, parasitoid of <i>Cinara</i> aphids on <i>Juniperus</i> .	<i>P. cupressobii</i> Stary
5.	Antennae with 21 antennomeres, R1 vein longer than pterostigma (0.80–0.85 pterostigma/R1 ratio) (Fig. 2e), body light brown to yellow.	<i>P. similis</i> Stary
-	Antennae with 19–20 antennomeres, R1 vein subequal to shorter than pterostigma (0.95–1.22 pterostigma/R1 ratio).	6.
6.	Tergite I 3.2 times as long as wide at spiracle level, R1 vein shorter than pterostigma length (1.20–1.22 ratio), propodeum with irregular lateral carinae (Fig. 2c), parasitoid of <i>Cinara</i> aphids on <i>Juniperus</i> .	<i>P. juniperorum</i> Stary
-	Tergite I more elongated, 3.5 times as long as wide at spiracle level, R1 vein subequal to pterostigma length (0.95–1.0 ratio), propodeum with transverse carinae only (Fig. 2d), parasitoid of <i>Cinara</i> aphids on <i>Pinus</i> .	<i>P. silvestris</i> Stary
7.	First flagellomere (F1) 2.4–2.7 times as long as wide, ovipositor sheath very narrow (Fig. 1b)	<i>P. unilachni</i> Gahan
-	First flagellomere (F1) 1.4–2.0 times as long as wide, ovipositor sheath stouter, curved upwards, narrowing towards apex or parallel sided.	8.
8.	First flagellomere (F1) 1.7–2.0 times as long as wide, ovipositor sheath long, stout, curved upwards, parallel sided, blunt at apex (Fig. 2c), parasitoid of <i>Cinara</i> aphids on <i>Picea</i>	<i>P. picta</i> Haliday
-	First flagellomere (F1) very short, 1.4–1.5 times as long as wide, ovipositor sheath curved, narrowing towards the apex (Fig. 2d), specialized parasitoid of cedar aphid <i>Cinara cedri</i> on <i>Cedrus</i>	<i>P. anatolica</i> Michelena, Assael & Mendel

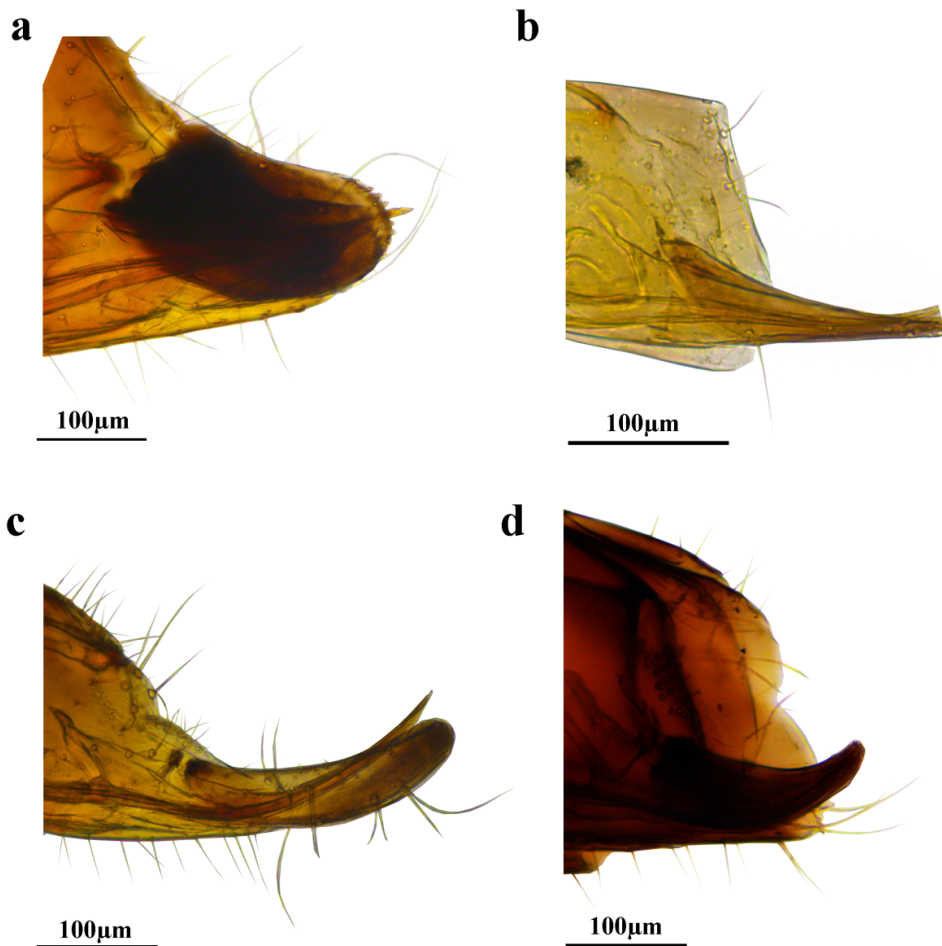


Figure 1. Different types of ovipositor sheaths: a – *P. cupressobii*, b – *P. unilachni*, c – *P. picta*, d – *P. anatolica*.

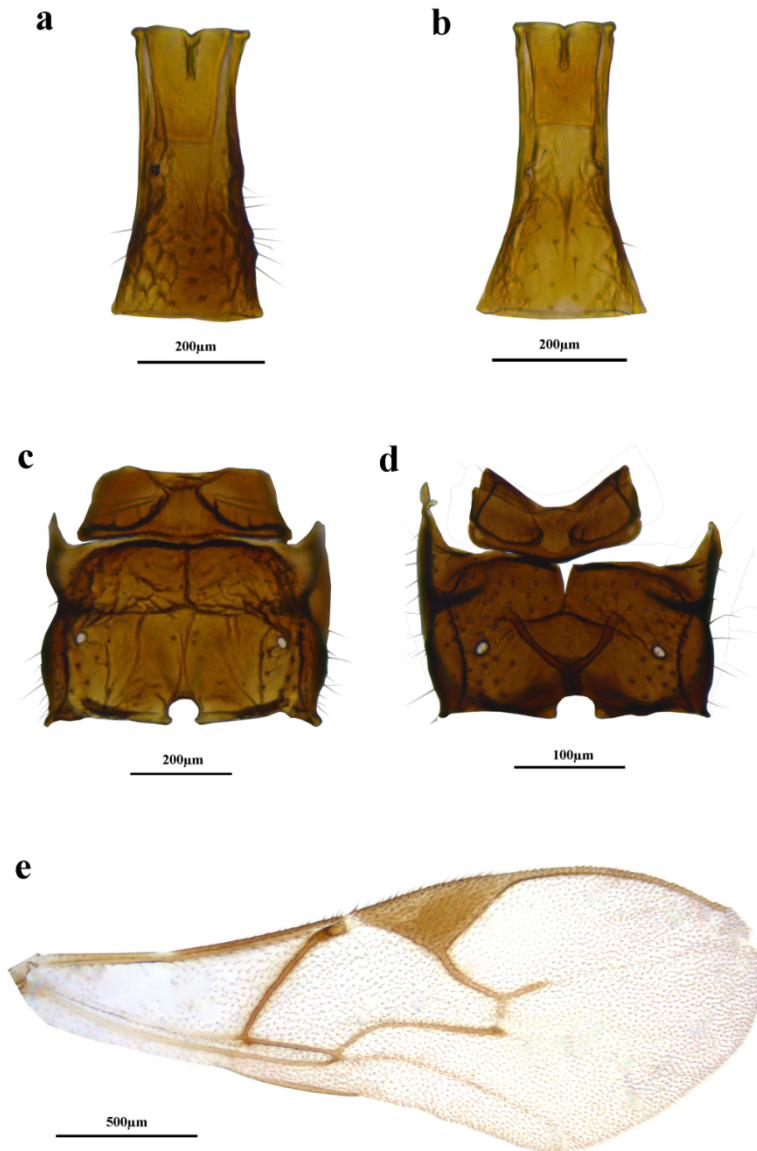


Figure 2. Tergite I, dorsal view: a – *P. piceaecollis*, b – *P. cupressobii*; propodeum, dorsal view: c – *P. juniperorum*, d – *P. silvestris*; forewing, e – *P. similis*.

Discussion

The first revision of the genus (under the name *Paraphidius*) was conducted by Starý in 1960 (Starý, 1960). In this study, the author emphasized the need for further revision and anticipated that many new species would be identified as more material was collected. As already highlighted, despite over 40 new species being described since then, a comprehensive modern revision is still lacking after 60 years.

Following extensive sampling focused exclusively on aphid parasitoids found on conifer plants, three new species previously unreported in the Serbian fauna have been identified, *P. silvestris*, *P. juniperorum*, and *P. similis*. Additionally, we report two new trophic associations, both involving *Pinus peuce* Griseb. (Macedonian pine), a Balkan endemic and tertiary relic species (Stojičić *et al.*, 2024): *P. similis* and *P. piceaecollis*, both reared from *Cinara* aphids. Specimens of *P. juniperorum* and *P. cupressobii* were exclusively found parasitizing *Cinara* aphids on *Juniperus*, demonstrating a strong specialization towards the subgenus *Cupressobium*. Moreover, *P. picta* was reared solely from aphids found on *Picea abies* (*C. costata* and *Cinara* sp.). Specimens of *P. anatolica* were collected from *Cedrus* trees cultivated for ornamental purposes in urban environments. This parasitoid species specializes in attacking aphids belonging to the subgenus *Cedrobium* and was reared from *Cinara cedri*. Interestingly, although multiple samples of aphid colonies from *Abies alba* were collected, no parasitoids emerged.

Preliminary molecular analysis of the barcoding region (COI) revealed two additional *Pauesia* species in Serbian fauna (unpublished results). These species were reared from *Cinara* sp. on *Pinus sylvestris* and *Picea abies* and are currently identified only to the genus level. Further sampling efforts to obtain additional material, along with the integration of molecular analysis, are needed to accurately assess the true diversity of *Pauesia* in Serbia.

Acknowledgments

We thank the Serbian Academy of Sciences and Arts for permission to use a portion of the record list, which part of a broader study that has already been published in Serbian in Cyrillic (Tomanović *et al.* 2021). The article is supported by the Serbian Ministry of Science and Education (451-03-47/2023-01/ 200178; 451-03-47/2023-01/ 200124) and the Serbian Academy of Sciences and Arts (grant no. F131). We are also grateful to the Rufford Foundation for awarding authors Korana Kocić and Jelisaveta Čkrkić two small conservation grants that funded the sampling of most of the presented material. We are grateful to Professor Olivera Petrović Obradović from the Faculty of Agriculture, University of Belgrade, for aphid identification.

References

- Davidian, E. M. (2015). Parasitoid wasps of the subgenus *Pauesia* Quilis s. str. (Hymenoptera, Aphidiidae) from Russia and neighboring countries. *Entomological Review*, 95, 500-506. <https://doi.org/10.1134/S0013873815040120>
- Kamanga-Thole, G., Mwase, W., Kamoto, J., Chilima, C., Missanjo, E. (2021) Effectiveness of *Pauesia juniperorum* in controlling population of cypress aphids in Malawi. *Journal of Global Ecology and Environment*, 13(2), 27-34.
- Kavallieratos, N. G., Tomanović, Ž., Starý, P., Athanassiou, C. G., Sarlis, G. P., Petrović, O., Niketić, & Veroniki, M. (2004). A survey of aphid parasitoids (Hymenoptera: Braconidae: Aphidiinae) of Southeast Europe and their aphid - plant associations. *Applied Entomology and Zoology*, 39, 527-563.
- Kavallieratos, N. G., Tomanović, Ž., Petrović, A., Kocić, K., Kaiser, M., & Starý, P. (2016). Parasitoids (Hymenoptera: Braconidae: Aphidiinae) of aphids feeding on ornamental trees in Southeastern Europe: key for Identification

- and tritrophic associations. *Annals of the Entomological Society of America*. 109. saw003. 10.1093/aesa/saw003.
- Kfir, R., Kirsten, F., Van Rensburg, N. J. (1985) *Pauesia* sp. (Hymenoptera: Aphidiidae): A parasite introduced into South Africa for biological control of the black pine aphid, *Cinara cronartii* (Homoptera: Aphididae). *Environmental Entomology*, 14(5), 597-601. <https://doi.org/10.1093/ee/14.5.597>
- Schneider, C. A., Rasband, W. S., & Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years of image analysis. *Nature Methods*, 9(7), 671–675. 10.1038/nmeth.2089
- Sharkey, M. J., & Wharton, R. A. (1997). Morphology and terminology. In Wharton, R.A., Marsh, P.M., and Sharkey, M.J. (eds), *Manual of the New World genera of the family Braconidae* (Hymenoptera). Washington, DC, USA: The International Society of Hymenopterists, 19-37.
- Sopow, S., Wardhaugh, C., Turner, R., Gresham, B., Sutherland, R., Woodall, G., Withers, T. (2021) Host specificity testing of *Pauesia nigrovaria* (Hymenoptera: Braconidae: Aphidiinae) for classical biological control of *TuberoLachnus salignus* (Hemiptera: Aphididae: Lachninae) in New Zealand. *BioControl*, 66, 739-751. <https://doi.org/10.1007/s10526-021-10107-5>
- Starý, P. (1960). A taxonomic revision of the European species of the genus *Paraphidius* Stary, 1958 (Hymenoptera, Braconidae, Aphidiinae). *Acta Faunistica Entomologica Musei Nationalis Prague*, 6, 5-44.
- Tomanović, Ž., Žikić, V., & Petrović, A. (2021). *Fauna of parasitoid wasps (Hymenoptera, Braconidae, Aphidiinae) of Serbia*. Serbian Academy of Sciences and Arts, Monographs, Book 15, 262 pp [in Serbian].
- Yu, D. S. K., van Achterberg, C., & Horstmann, K. (2016). *Taxapad, Ichneumonoidea*. Vancouver. <http://www.taxapad.com>

PAUESIA QUILIS, 1931 (HYMENOPTERA, BRACONIDAE; APHIDIINAE) У СРБИЈИ: ТРИ НОВЕ ВРСТЕ ЗА ФАУНУ СРБИЈЕ И КЉУЧ ЗА ИДЕНТИФИКАЦИЈУ ВРСТА

КОРАНА КОЦИЋ, ЈЕЛИСАВЕТА ЧКРКИЋ, АНЂЕЉКО ПЕТРОВИЋ И ЖЕЉКО ТОМАНОВИЋ

Извод

Pauesia је један од већих родова унутар потфамилије Aphidiinae, и обухвата око 80 врста распрострањених широм холарктичког региона. Упркос таквом дивезитету и дистрибуцији, савремена свеобухватна ревизија рода не постоји. У Србији је тренутно забележено шест врста овог рода. Резултати наше студије откривају три врсте нове за фауну Србије: *Pauesia similis* Stary, 1966, *Pauesia juniperorum* (Stary, 1960) и *Pauesia silvestris* (Stary, 1960). У овој студији су представљени и подаци о локацијама сакупљања и тритрофичке асоцијације анализираних материјала и дат је кључ за идентификацију женки рода *Pauesia* које су забележене у Србији.

Received: July 15th, 2024
Accepted: October 3rd, 2024