Received: 28 June 2023 | Revised: 11 August 2023 | Accepted: 13 August 2023

DOI: 10.1111/jen.13182

SHORT COMMUNICATION

JOURNAL OF APPLIED ENTOMOLOGY WILEY

First record of *Leptopilina japonica* Novković & Kimura, 2011 (Hymenoptera: Figitidae) in Germany, a parasitoid of the Spotted Wing Drosophila *Drosophila suzukii* (Matsumura, 1931) (Diptera: Drosophilidae)

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Abstract

Two years after the first European record in Italy, we report the first occurrence of the parasitoid wasp *Leptopilina japonica* Novković & Kimura, 2011 (Hymenoptera: Figitidae) in Germany. The species is a larval-pupal parasitoid of *Drosophila suzukii* (Matsumura, 1931) (Diptera: Drosophilidae), which is a widespread invasive and economically important pest of soft-skinned fruit. In total, we found 29 specimens of *L. japonica* in five different locations in southern and western Germany in the years 2021, 2022 and 2023. We examined the specimens morphologically and generated their DNA barcodes for identification. In three of the locations, *L. japonica* was sampled from raspberries. In two locations, *L. japonica* was caught in two and three consecutive years, respectively, which indicates adventive establishment. As *D. suzukii* and *L. japonica* originate from the same region in Asia, the possible establishment of *L. japonica* could be a case of unintentional biological control in Germany. In addition to this first record in Germany, we present a diagnosis of *L. japonica* to distinguish the species from the rest of the European *Leptopilina* fauna.

KEYWORDS
Drosophila suzukii, invasive species, *Leptopilina japonica*, neozoa, parasitoid, unintentional biological control

1 | INTRODUCTION

The spotted wing drosophila (SWD, *Drosophila suzukii* (Matsumura, 1931) (Diptera: Drosophilidae)) originates from Southeast Asia and was first detected in Europe in Spain in 2008 (Calabria et al., 2012). It was discovered in Germany 3 years later (Heuck, 2012; Vogt et al., 2012) and had spread across most of the continent by 2012 (Cini et al., 2014). The species is a pest of many economically important soft-skinned fruit; its host plant range covers cherry, raspberry and blueberry, but also a wide variety of wild non-crop hosts, which allows SWD to switch between hosts in the course of a year (Kenis et al., 2016). Under suitable conditions, the generation time is below 2 weeks, leading to rapid population growth (Tochen et al., 2014). Management strategies of SWD focus mostly on cultural practices (e.g. exclusion netting) and insecticides, but parasitoids, both from Southeast Asia and from the invaded areas, are under investigation for their potential in biological control (Wang et al., 2020).

In Europe, SWD is most commonly attacked by the native pupal parasitoids *Trichopria drosophilae* (Perkins, 1910) (Hymenoptera:

Diapriidae) and *Pachycrepoideus vindemmiae* (Rondani, 1875) (Hymenoptera: Pteromalidae) (Englert & Herz, 2016; Knoll et al., 2017; Kremmer et al., 2017) and these are currently explored for use in augmentative biological control of SWD in Germany (Eben et al., 2022). European larval-pupal parasitoids of SWD have very low reproduction rates due to host resistance and lead to low levels of mortality of the host (Kruitwagen et al., 2021; Poyet et al., 2013); these species may fail to attack SWD when it is offered as a host (Chabert et al., 2012).

In its area of origin, SWD is most frequently parasitized by the two specialized larval-pupal parasitoids *Leptopilina japonica* Novković & Kimura, 2011 and *Ganaspis brasiliensis* (Ihering, 1905) (Hymenoptera: Figitidae) (Daane et al., 2016; Girod et al., 2018). Both species have followed their host to western parts of North America and have been present there since 2016 (*L. japonica*) and 2019 (*G. brasiliensis*) (Abram et al., 2020). They have since established adventive populations (Beers et al., 2022). *G. brasiliensis* is also found in several Central and Southern American countries (Buffington & Forshage, 2016; Gallardo et al., 2022; Gonzalez-Cabrera et al., 2020) and has been released since 2021 as part of a classical biological control programme in Italy (Fellin et al., 2023). *L. japonica* is less widely distributed and the only report outside of its area of origin in Southeast Asia (Novković et al., 2011) or North America (Abram et al., 2020) is from Italy in 2019 (Puppato et al., 2020).

In this study, we report the presence of *L. japonica* in Germany for the first time, which is also the northernmost record of the species in the world. This arrival of *L. japonica* may open the possibility to enhance natural regulation of SWD in the future.

2 | MATERIALS AND METHODS

We analysed specimens from five different locations in western and southern parts of Germany, details on locations and collection methods are given in Table 1. From three of the locations, wasps were reared from drosophilid-infested berries; in one of them, the berries were exclusively infested by SWD. The collections in Veitshöchheim were conducted to monitor the parasitoid complex of SWD in Germany.

Sequences of the CO1 barcode region were obtained from five specimens (Table 1) using standard procedures at Advanced Identification Methods (AIM, Leipzig, Germany, see Morinière et al., 2015) and the Leibniz Institute for the Analysis of Biodiversity Change (LIB, Museum Koenig Bonn, Germany, see Jafari et al., 2023 for lab protocol, and Astrin & Stüben, 2008 for the LCO1490-JJ forward- and the HCO2198-JJ reverse-primer). We combined our CO1 barcode sequences with those of *Leptopilina* specimens deposited at the DROP Database (Lue et al., 2021), including the sequences published together with the description of *L. japonica* (Novković et al., 2011). Using Geneious (vers. 7.1.9, Biomatters Ltd.), we aligned sequences with MUSCLE and generated a neighbour-joining tree (Tamura-Nei). Based on this tree, we evaluated conspecificity of the sampled specimens and those with data deposited at DROP on their distance-based clustering.

In addition to CO1 barcode analysis, we morphologically examined barcoded specimens and 24 additional specimens from the same localities using a Leica M205C stereomicroscope. We used the latest treatments of the genus for the Western Palearctic region (Forshage & Nordlander, 2008; Nordlander, 1980; Novković et al., 2011; van Alphen et al., 1991), including the relevant terminology. Our diagnosis of *L. japonica* is based on information from these sources and verified with specimens of all included taxa, except *L. australis* (Belizin, 1966), since no specimen of that species was available to us, but which can be distinguished from *L. japonica* through literature alone.

For imaging, we used a Canon EOS 7D® camera mounted on a P-51 Cam-Lift (Dun Inc.). The image stacks were evaluated in Adobe LightRoom® vers. 5.6 and stacked with Helicon focus® vers. 8.2.2. We aligned and enhanced (adjusting luminescence values, unsharp masking), the images in GIMP 2.10 and finally composed Figure 2 with Scribus vers. 1.4.8.

3 | RESULTS

We found 29 *L. japonica* from three different years (2021–2023) and five different locations in Germany, spanning from Latitude 49.8–50.7 and Longitude 7.1–8.2. (Figure 1).

The genus *Leptopilina* Förster, 1869 is recognized by having a postpetiolar rim and a dorsally interrupted ring of hairs on the metasomal base. The European fauna consists of the species *L. australis*, *L. boulardi* Barbotin, Carton & Keiner-Pillault, 1979, *L. clavipes* (Hartig, 1841), *L. fimbriata* (Kieffer, 1901), *L. heterotoma*, *L. longipes* (Hartig, 1841) (Nordlander, 1980; van Alphen et al., 1991) and the hereby reported *L. japonica*.

The species *L. japonica* is diagnosed against the remaining European species by having no row of long hairs along the sides of the mesoscutum (white arrow Figure 2a; opposing *L. australis* & *L. clavipes*: Nordlander, 1980: 437, figure 12), the mesoscutellar foveae being wider than long (green arrow Figure 2b; about as wide as long or longer in *L. longipes*: Nordlander, 1980: 445, figure 38 and *L. fimbriata*: Nordlander, 1980: 445, figure 39) and the dorsal mesoscutellar surface being punctate reticulate (in some males foveate-reticulate sculpture intermixing with punctuation lateromedially) (blue arrow Figure 2b; dorsal mesoscutellar surface with at most little punctuation anteriorly and/or posteriorly, but lateromedially never punctate in both sexes of *L. heterotoma*: Figure 2d & *L. boulardi*: Nordlander, 1980: 445, figure 46).

The result from analysis of the nucleotide sequence data is congruent with the results from the morphological identification. Our sequences match the reference sequences of *L. japonica* from DROP with 97.8% to 100% similarity (excl. the cluster around the TP strain, since none of our sequences fell within that cluster and the specimens were described as separate subspecies of *L. japonica*). The minimum similarity among the sequences in the DROP dataset (i.e. alignment without our sequences) is 98.2%.

TABLE 1 List of all specimens examined both morphologically and molecularly.

Locality	Collection method	Specimen ID	Sex	Date	Sequencer	DROP sequence ID	BOLD process ID	Sequence length (bp)	Depository
Bonn (North Rhine-Westphalia) within the city in the garden of the Museum Koenig (ZMFK)	sweep net and yellow pan traps	ZFMK-TIS-2632469	♂	Aug 2021	BGI	614	GBHYG1651-23	658	ZFMK
		ZFMK-TIS-2632471	♀	Aug 2021	BGI	615	GBHYG1653-23	658	ZFMK
		ZFMK-TIS-2632472	♂	Aug 2021	BGI	616	GBHYG1654-23	588	ZFMK
		ZFMK-TIS-2637731, ZFMK-TIS-2637732	♂	June 2022	-	-	-	-	ZFMK
		ZFMK-TIS-2635306	♀	Oct 2022	BGI	617	GBHYG1714-23	658	ZFMK
		ZFMK-HYM-00039549	♀	June 2023	-	-	-	-	ZMFK
Dossenheim (Baden-Württemberg) experimental field of the Julius Kühn Institute (JKI)	reared from SWD pupae in raspberries under laboratory conditions (23°C, 70% relative humidity) for 6 weeks	21_07040102	♀	Sept 2021	AIM	-	GBBAD001-23	251	JKI
		SMNS_Hym_Hym_014629	♀	Oct 2021	-	-	-	-	SMNS
		SMNS_Hym_Hym_014630	♂	Sept 2021	-	-	-	-	SMNS
Neustadt a.d.W. (Rhineland-Palatinate) experimental field of the Dienstleistungszentrum Ländlicher Raum Rheinpfalz (DLR)	live catches from raspberries	ZFMK-TIS-2641361	♀	Oct 2022	-	-	-	-	ZFMK
		22_N_Lj1, 22_N_Lj2, 22_N_Lj3	♀	Oct 2022	-	-	-	-	JKI
Reinheim (Hesse) garden of a residential area	reared from drosophilid-infested raspberries	21_R_Lj1	♀	Sept/Oct 2021	-	-	-	-	Coll. R. Weber
		ZFMK-TIS-2641360	♀	Sept/Oct 2022	-	-	-	-	ZFMK
Veitshöchheim (Bavaria) experimental field of the Bayerische Landesanstalt für Weinbau und Gartenbau (LWG)	reared from SWD-infested blackberries under outdoor conditions until June 2023	ZFMK-HYM-00039555 -00039560	♀	Sept 2022	-	-	-	-	ZMFK
		23_V_Lj7-Lj13	♀	Sept 2022	-	-	-	-	JKI

Note: Depositories are abbreviated as follows: Museum Koenig Bonn, Germany (ZFMK), Julius Kühn Institute in Dossenheim, Germany (JKI), State Museum of Natural History, Stuttgart, Germany (SMNS).

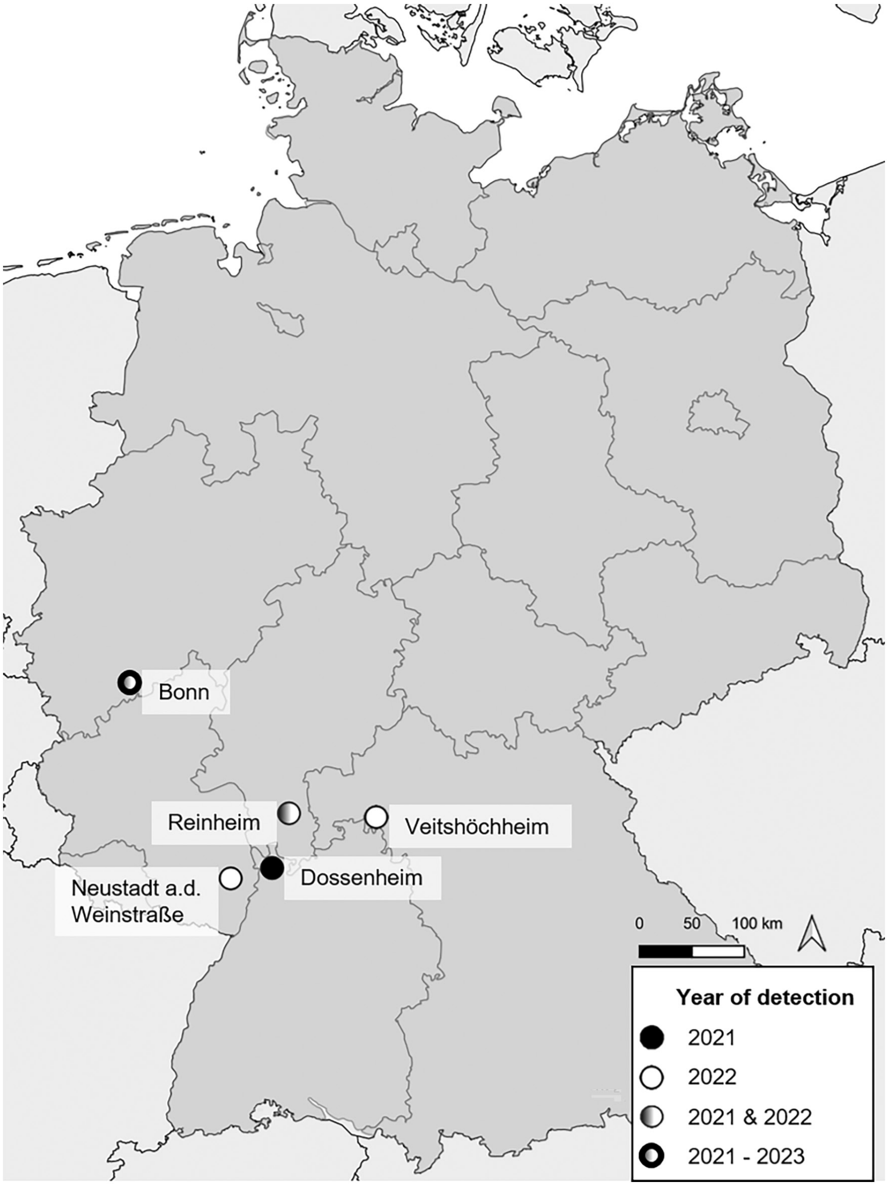

FIGURE 1 Records of *Leptopilina japonica* in Germany.

We deposited all sequence data in the DROP database (http://doi.org/10.5281/zenodo.4519656; Lue et al., 2021) and in BOLD (Ratnasingham & Hebert, 2007).

4 | DISCUSSION

The same way that pests spread without intentional human interference, their natural enemies may spread as well (Weber et al., 2021). In the case of non-native parasitoid Hymenoptera in Europe, 45% originate from unintentional introductions (Weber et al., 2021). These unintentional introductions may substantially reduce pest populations, as in the case of *Aphelinus certus* Yasnosh, 1963 (Hymenoptera: Aphelinidae) in the United States (Kaser & Heimpel, 2018), or serve as unintended test area for a later classical biological control programme, as in the case of *Trissolcus japonicus* (Ashmead, 1904) (Hymenoptera: Scelionidae) in Italy (Falagiarda et al., 2023).

We are not aware of any previous detections of *L. japonica* in Germany, and it was also not found in an earlier survey of SWD parasitoids (Englert & Herz, 2016). However, because *L. japonica* was discovered in 2021 at three different locations up to 220 km apart, it is likely that the species is even more widespread or has been (accidentally) introduced multiple times. In two of the locations, *L. japonica* was found in consecutive years, which may indicate that adventive populations are established. The specimens in Germany, especially in Bonn, represent, to the authors' knowledge, the northernmost findings of *L. japonica* (Latitude 50.7). This is possibly due to the mild mesoclimate of the Rhine valley and the favourable conditions in the urban environment. The small number of recorded specimens in this study is linked to the unsystematic type of collecting efforts and probably does not relate to actual population sizes. Although not restricted to the *Drosophila melanogaster* species group, the known host range of *L. japonica* is relatively narrow (Daane et al., 2021; Kimura & Novković, 2021;

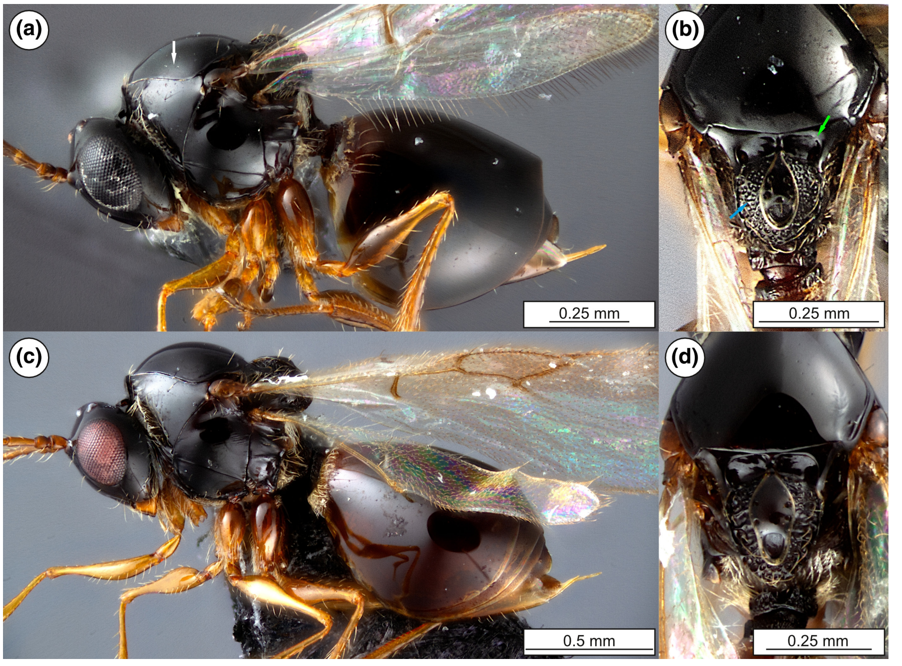

FIGURE 2 (a, b) *Leptopilina japonica* habitus (lateral view, a) and mesoscutellum (dorsal view, b). The arrows indicate diagnostic characters mentioned in the diagnosis. (c, d) *L. heterotoma* habitus (lateral view, c) and mesoscutellum (dorsal view, d).

Wang et al., 2020), so its establishment in Germany poses limited, if any, risk to non-target organisms in the native fauna. In contrast, it will be all the more interesting to what extent the natural regulation of invasive SWD, which also occurs outside crops in wild berry fruits, is enhanced by the arrival of this effective larval-pupal parasitoid. Further investigations are required to monitor the distribution and abundance of *L. japonica* to assess its impact as an agent of unintentional biological control as well as its impact on non-target species.

AUTHOR CONTRIBUTIONS

Jakob Martin: Conceptualization; writing – original draft; investigation. **Annette Herz:** Conceptualization; writing – original draft. **Jonathan Vogel:** Conceptualization; investigation; writing – original draft. **Ralph S. Peters:** Conceptualization; writing – original draft.

ACKNOWLEDGEMENTS

We thank Camilla Englert and Reinhard Weber for collecting and providing specimens from Neustadt and Reinheim respectively; Alexander Zimmermann for allowing access to the LWG experimental field in Veitshöchheim and Sophie Reiher for collecting the specimens from LWG, the OEP Arthropod course of 2021 for collecting and providing the specimens collected in the ZFMK garden 2021 and Tobias Salden and Inci L. Baez for providing specimens collected in 2022 and 2023, respectively, in the ZFMK garden. J. Vogel and R.S. Peters are supported by a grant from the Federal Ministry of Education and Research (BMBF), Berlin, Germany (FKZ 16LI1901A), for the project 'GBOL III: Dark Taxa'. J. Martin is supported by funds of the Federal Ministry of Food and Agriculture (BMEL) based on a decision of the Parliament of the Federal Republic of Germany via the Federal Office for Agriculture and Food (BLE) under the innovation support programme (FKZ 2818805A19). Open Access funding enabled and organized by Projekt DEAL.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The specimens at ZMFK, JKI and SMNS (Table 1) are available at the respective repositories. We deposited all sequence data openly available in the DROP database (http://doi.org/10.5281/zenodo.4519656; Lue et al., 2021) and in BOLD (http://www.boldsystems.org/; Ratnasingham and Hebert, 2007).

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How to cite this article: Martin, J., Vogel, J., Peters, R. S., & Herz, A. (2023). First record of *Leptopilina japonica* Novković & Kimura, 2011 (Hymenoptera: Figitidae) in Germany, a parasitoid of the Spotted Wing Drosophila *Drosophila suzukii* (Matsumura, 1931) (Diptera: Drosophilidae). *Journal of Applied Entomology*, *147*, 1067–1073. https://doi.org/10.1111/jen.13182