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SHORT COMMUNICATION

Egg sac parasitism: how important are parasitoids in the range expansion of the wasp spider *Argiope bruennichi*?

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Abstract. During recent decades, the wasp spider, *Argiope bruennichi* (Scopoli, 1772), has expanded relatively quickly towards north Europe. As a consequence of its spreading, it is newly exposed to various factors of selection. We studied the impact of egg sac parasitoids on the mortality of *A. bruennichi* in three regions differing in climate conditions and time of settling by this spider. Parasitism of wasp spider egg sacs was relatively low (0–3.9%) and no significant differences between studied regions were found. One primary parasitoid, *Tromatobia ornata,* was reared; in approximately 60% of these parasitized cocoons, the entire content of the egg sac was destroyed.

Keywords: Pseudohyperparasitoid, host, *Tromatobia ornata*, *Pediobius brachycerus*

The wasp spider, *Argiope bruennichi* (Scopoli, 1772) is a Palaearctic species that has been expanding northward from the Mediterranean (Kumschick et al. 2011); its European range currently also includes Scandinavia (Jonsson & Wilander 1999; Bratli & Hansen 2004; Koponen et al. 2007). In Poland, the wasp spider was first recorded in 1935 (Urbański 1935), and, for several decades, its area was restricted only to the west and south-eastern parts of the country. Since the 1990s, *A. bruennichi* has rapidly spread towards the north. Now, the wasp spider occurs throughout Poland and is numerous everywhere, except for the higher elevation of the mountains (Wawer 2012). The expansion of some European species to the north may be connected with global warming (e.g., Hughes 2000; Walther et al. 2002; Hickling et al. 2006). In the case of the thermophilous wasp spider, this may be one of the most important factors (Ivinskis et al. 2009), but other factors may also favor its spreading, e.g., prolongation of the growing season, increasing the area of fallow lands or transport intensification causing passive dispersion (Guttmann 1979).

As a consequence of expansion, *A. bruennichi* is exposed to new factors of selection (Leborgne & Pasquet 2005). Natural enemies have a significant mortality impact on local spider populations (Polis et al. 1998), and parasitoids might be the most important of these (Foelix 2011). Among parasitoids, there are some that develop individually within eggs (e.g., Scelonidae), or feed on spider egg masses (e.g., Mantispidae, Ichneumonidae, Phoridae), as well as ectoparasitoids (e.g., Ichneumonidae) and endoparasitoids (e.g., Acroceridae) of post-embryonic spiders—both spiderlings and mature spiders (Austin 1985; Fitton et al. 1987; Schlinger 1993; Allard & Robertson 2003; Finch 2005). The mortality of eggs and spiderlings is considered to be the most significant factor regarding the spider life cycle (Topping 1997). Parasitoids that are associated with the wasp spider are species of the Ichneumonidae and Eulophidae (Hymenoptera). Among the ichneumonid wasps, *Tromatobia ornata* (Gravenhorst, 1829) from the Pimplinae (Rollard 1985, 1990) and *Buathra tarsoleucos* (Schrank, 1781) and *Thaumatogelis gallicus* (Seyrig, 1928) from the Cryptinae have been recorded as parasitizing *A. bruennichi* (Fahringer 1922; Schwarz 2001). These species develop by feeding on cocooned spider eggs, but none of them are specific to *A. bruennichi* (Fitton et al. 1987; Yu et al. 2012). The parasitic wasp from the Eulophidae, *Pediobius brachycerus* (Thomson, 1878) is an obligatory hyperparasitoid (secondary parasitoid) of spider egg sacs, which necessarily parasitizes a spider's primary parasitoids, including some species of ichneumonid parasitoid wasps (Fitton et al. 1987; Kostro-Ambroziak & Wawer 2015). Here we studied the influence of egg sac parasitoids on the mortality of *A. bruennichi* in regions differing by time of settling of this spider and climatic conditions, based on populations from Poland.

Investigations were carried out from 2011 to 2013. Egg sacs of *A. bruennichi* were collected from nine localities in three regions of Poland: the Suwalki Lake District (SLD1: 54°8′14.88″N, 22°55′58.94″E; SLD2: 54°8′32.29″N, 22°50′55.37″E; SLD3: 54°5′25.23″N, 22°59′12.55″E), the Mazovian Lowland (ML1: 52°19′5.39″N, 20°52′32.29″E; ML2: 52°22′46.67″N, 20°47′47.04″E; ML3: 52°0′29.66″N, 21°22′12.58″E), and the Sandomierz Valley (SV1: 50°10′38.01″N, 21°43′12.09″E; SV2: 50°10′48.08″N, 21°42′31.02″E; SV3: 50°8′57.20″N, 21°40′36.67″E) (Fig. 1).

In south-east Poland, the first individuals of *A. bruennichi* were discovered in the 1960s (the Low Beskids) (Bednarz 1966). In the Mazovian Lowland, *A. bruennichi* was observed for the first time in 1998 (Kajak & Łuczak 2003). At that time, this species was considered to be rare and endangered in Poland, which led to its protection by law. In northern Poland (the Suwalki Lake District), the wasp spider was observed for the first time in 2005 (W. Wawer, unpubl.). Meanwhile, recent years have brought a wave of expansion of unusual intensity, causing species dispersal and establishment across virtually the entire country. The number of known locations doubled from 1990 to 2007 (Wawer 2014). The three regions mentioned above (SLD, ML, SV) differ in climatic conditions — the region furthest to the north is the coldest and probably due to this factor, it is characterized by fewer *A. bruennichi.*

Egg sacs overwintered in natural conditions on plant leaves, ca. 20 cm above the ground, in open areas, mainly in meadows and on agricultural wastelands. In April, they were collected and transported in a plastic jar and kept at a constant temperature of 8 °C until the dissection began. The egg sacs were opened at the end of April. Each egg sac was examined under a microscope and cut by medical scissors. Parasitized egg sacs were stored at room temperature on a piece of cotton wool in a plastic jar (50 ml), and every day, a few drops of water were added to preserve humidity. The adult parasitic wasps emerged after about 10 days (April/May).

Adult wasps were identified by morphological characteristics with reference to the taxonomic literature (Bouček 1965; Fitton et al. 1988). Additionally, pupal cases of parasitoids were confirmed by DNA barcode sequences. Reference Sequences (RefSeq) were obtained in this study. Genomic DNA was extracted using a Genomic

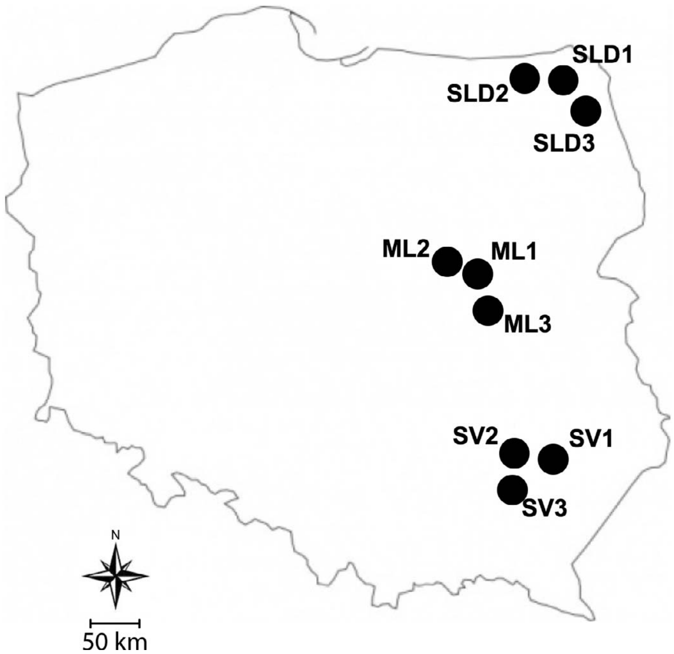

Figure 1.—Distribution of localities in Poland where the egg sacs of *Argiope bruennichi* were collected: the Suwalki Lake District (SLD), the Mazovian Lowland (ML) and the Sandomierz Valley (SV).

Mini Kit (A&A Biotechnology). The primers LepF1 (5′-ATTCAACCAATCATAAAGATATTGG-3′) and LepR1 (5-TAAACTTCTGGATGTCCAAAAAATCA-3′) were used for COI mtDNA fragment amplification (650 pz) (Smith et al. 2009). PCR consisted of an initial activation step at 95 °C for 15 min; 45 cycles of denaturation at 94°C for 30 sec, annealing at 52 °C for 90 sec and extension at 72 °C for 60 sec and a final extension at 60 °C for 30 min. The sequence data for the COI gene sequences were submitted to GenBank with the accession number KU870312. All voucher specimens are deposited in the collection of Museum and Institute of Zoology Polish Academy of Sciences.

In total, 560 egg sacs of *A. bruennichi* were examined. The degree of parasitism of wasp spider egg sacs was 3.9% (Table 1). In the central region ML, we noticed no infested egg sacs. No significant differences between the other two regions (SLD and SV) were found (Fisher's Exact test, $P = 0.21$). One primary parasitoid, *Tromatobia ornata*, was reared from 22 egg sacs and the secondary parasitoid *Pediobius brachycerus* from 11 egg sacs (Table 1). The former parasitoids were noticed as pupae (1–3 per sac) and the latter as larvae inside pupal casings (1–8 per sac). Only two egg sacs produced two or three individuals of *T. ornata*. The reproductive fitness of the *A. bruennichi* female was greater than zero in all cases of parasitization. In 41% of parasitized cocoons, an average of 148 living nymphs of spiders per egg sac were detected (SD = 100.1; range = 1–220). The unparasitized egg sacs either contained up to 642 living nymphs of the wasp spider, or were damaged, empty or with undeveloped embryos. Both in parasitized and unparasitized egg sacs, a portion of the spider eggs failed to develop (59% and 35.3%, respectively), a statistically significant difference (Chi-square test, $X^2{}_1 = 475.5$, $P < 0.001$).

The egg-sac of *A. bruennichi* is under maternal care for a few days (Leborgne & Pasquet 2005). The layer structure of the wasp spider cocoon protects eggs and spiderlings from fluctuating temperatures and desiccation, as well as acting as a mechanical barrier against parasitoids and parasites (Hieber 1985, 1992; Bergthaler 1995). *Tromatobia ornata* lays eggs into spider cocoons in reddish threads close to the outer layer (Rollard 1990). Parasitism of the egg sac of *A. bruennichi* by *T. ornata* in Poland is distinctly lower (0–3.9%) than in Germany (0–50%) (Sacher 2001) or France (8–44%) (Leborgne & Pasquet 2005), where *T. ornata* is also a main parasitoid in egg sacs of this spider species. The low degree of parasitism in Poland may be the effect of a shift in the time of oviposition of host and parasitoid caused by the recent spread of *A. bruennichi*. Rollard (1987) revealed that egg sacs were parasitized by *T. ornata* only by the time juveniles emerged. The spider embryos develop in approximately 2 to 3 weeks and nymphs hibernate during winter in the egg sac (Von Becker 1983; Rollard 1987). Because of this, females of *A. bruennichi* that lay their eggs early avoid parasitoids and have higher reproductive success (Leborgne & Pasquet 2005). Because *T. ornata* is relatively widespread in Poland, it is probable that here it parasitizes other hosts, both other spiders and moths (Yu et al. 2012), and the wasp spider is not a limiting factor for this parasitoid.

Argiope bruennichi lays on average 800 eggs per sac (Rollard 1985; Köhler & Schäller 1987; Miyashita 1996). Larvae of *T. ornata* feed on spider eggs and develop very quickly to fifth instars, and in this inactive stage they overwinter inside the spiders' egg sacs (Rollard 1985). Little is known about the phenology of this parasitoid from spring to autumn. Oehlke & Sacher (1991) indicated that *T. ornata* is univoltine, but based on the biology of its other hosts (Yu et al. 2012), e.g., *Nuctenea umbratica* (Clerck, 1757), it is highly probable that it is at least bivoltine. Although *T. ornata* is known as a gregarious parasitoid (Fitton et al. 1988; Sacher 1988, 2001), we recorded mainly single specimens of it in egg sacs. We also noticed that not all of the parasitized egg sacs were destroyed. Rollard (1985) indicated that total destruction of spider eggs occurred only when there was more than one larva of *T. ornata* in a cocoon. According to Cortés et al. (2000), *Tromatobia* sp., as a parasitoid of *Araneus granadensis* (Keyserling, 1864), also destroys only a portion of the contents of the egg sac.

We recorded *P. brachycerus* as a gregarious parasitoid inside the pupae of *T. ornata*. According to these data and the earlier suggestion of Fitton et al. (1987) that *P. brachycerus* attacks the primary parasitoid during the pupal stage, we think it should be labelled as a pseudohyperparasitoid. In contrast to hyperparasitoids, which parasitize the larvae of other parasitoids while they are feeding on or in the primary host, pseudohyperparasitoids attack the primary parasitoid after it has completed feeding on its host (Quicke 2015). Of course, detailed studies on the biology of this parasitoid wasp are needed. *Pediobius brachycerus* is a parasitoid of some species of Ichneumonidae which parasitize spider eggs (Kostro-Ambroziak &

Table 1.—Egg sac parasitism of *Argiope bruennichi* in three regions differing in climate conditions and time of settling by the spiders: the Suwalki Lake District (SLD), the Mazovian Lowland (ML) and the Sandomierz Valley (SV).

Region	N of studied egg sacs	N and (%) of egg sacs parasitized by *T. ornata*	N and (%) of *T. ornata* pupae parasited by *P. brachycerus*
Suwalki Lake District (SLD)	220	15 (6.8%)	6 (33.3%)
Mazovian Lowland (ML)	220	0	0
Sandomierz Valley (SV)	120	7 (5.8%)	6 (75%)
Total	560	22 (3.9%)	12 (46.15%)

Wawer 2015), but *T. ornata* is recorded for the first time as its secondary host.

To summarize, in our study the overall mortality of *A. bruennichi* induced by its egg parasitoids was relatively low. Additionally, a high level of undeveloped eggs both in the parasitized and unparasitized egg sacs suggests that other factors, such as unfavorable wintering conditions (temperature, humidity) or fungal penetration, may have a significant impact on the mortality and life history of this range expanding spider.

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