

PDF issue: 2025-06-11

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Oviposition behaviour by a spider -
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(Citation) Journal of Zoology,308(3):221-230

(Issue Date) 2019-07

(Resource Type) journal article

(Version) Accepted Manuscript

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https://hdl.handle.net/20.500.14094/90008174



| 1  | Oviposition behaviour by a spider-ectoparasitoid, Zatypota maculata, exploits the         |
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| 2  | specialised prey capture technique of its spider host                                     |
| 3  | Short title: Oviposition behaviour of Zatypota maculata                                   |
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#### 19 Abstract

20 Oviposition on or into a living animal is an arduous undertaking for parasitoids, including 21 in terms of counterattack or evasion by the host. Parasitoids of active spiders protected 22 by their own webs (polysphinctine spider-ectoparasitoids in the family Ichneumonidae) 23 need to avoid entanglement with the spider's web to gain access to the spider host for 24 oviposition. These circumstances have driven the evolution of offensive behaviours 25 highly adapted to the web architecture of each spider host. We discovered offensive oviposition behaviour in a member of the Polysphincta-group, Zatypota maculata, 26 parasitising a theridiid spider, Nihonhimea japonica, which constructs a "knockdown 3D 27 28 web". It consists of a non-viscid 3D structure above, with a retreat (a dead leaf) hung at the centre, and a non-viscid sheet below as a capturing device. When a prey insect is 29 30 knocked down and then trapped by the sheet, the spider immediately drops onto the underside of the sheet by penetrating the sheet to bite and wrap it. Zatypota maculata 31 32 exhibits two behaviours to deal with this type of web: 1) the wasp climbs the 3D cobweb, 33 creeps up slowly onto the spider's retreat, taking a long time so that the spider does not 34 escape, and finally enters the retreat to sting the spider (creeping-style); 2) the wasp dives from the outside of the web onto the sheet, as a knocked-down prey item would, to lure 35 the spider out, before stinging it at the moment of contact (diving-style). We discuss these 36 37 unique offensive behaviours and compare them with behaviours of other polysphinctines and spider-hunting aculeates. Ovicide by means of removal of pre-existing eggs was also 38 observed under experimental conditions. 39

Keywords: aggressive mimicry; behavioural plasticity; ectoparasitoid; ovicide;
oviposition behaviour; polysphinctine; knockdown 3D web, Theridiidae, Ichneumonidae.

## 43 Introduction

Oviposition on an active host obliges the parasitoid to face risks of counter-attack or 44 45 evasion by potential hosts (Greeny et al. 2012, Gross 1993), which at worst cause the parasitoid's death. For example, a braconid endoparasitoid of ants, Elasmosoma 46 47 luxemburgense Wasmann 1909, was witnessed being caught by a host ant worker (Gómez 48 Durán & van Achterberg 2011). These risks never happen in oviposition by phytophagous, 49 saprophagous, and even predatory arthropods because their oviposition is never on living animals, suggesting that the parasitoids' oviposition requires specific and offensive 50 oviposition behaviour to breach protective behaviours of host taxa. 51

52 Among hymenopteran parasitoids, there is a group parasitising active predatory spiders called the *Polysphincta* group of genera (Hymenoptera, Ichneumonidae; hereafter 53 54 polysphinctine). This is a monophyletic lineage ecologically characterised by being solitary koinobiont ectoparasitoids of spiders with high host specificity (Gauld & Dubois 55 56 2006, Matsumoto 2016) and currently comprises 25 genera and over 250 species 57 distributed all over the world (Matsumoto 2016, Yu et al. 2016). To oviposit upon spider 58 host protected by their own webs, polysphinctines must have evolved ways of offensive (i.e. approaching and attacking) oviposition behaviour that is highly adapted to the web 59 architecture of each spider host. 60

In fact, the offensive oviposition behaviours of polysphinctines have been documented in several species, which exhibited highly specific strategies to deal with the web architecture of each spider host (Eberhard 2000, Gonzaga & Sobczak 2007, Iwata 1942, Kloss *et al.* 2016, Matsumoto 2009, Takasuka & Matsumoto 2011b, Takasuka *et al.* 2009, Weng & Barrantes 2007). Such behavioural records provide us with invaluable clues in discussing host shifts and speciation history of polysphinctines because oviposition is the initial event of new host utilisation. However, data are still very scarce
because of the rarity of witnessing these behavioural events *in situ* and the difficulty in
evoking them *in vitro*, despite there being over 250 described polysphinctine species.

The polysphinctine genus Zatypota is the largest of the genus-group, with 50 70 71 described species (Yu et al. 2016), with members in general parasitising spiders of the family Theridiidae (cf. Matsumoto & Takasuka 2010) which typically construct three-72 73 dimensional cobwebs that exclude 'sneaking' predators of spiders and kleptoparasites (spiders that steal prey or usurp webs) (Murphy & Roberts 2015) (a minority of Zatypota 74 species are parasitoids of the families Araneidae, Linyphiidae, and Dictynidae; see Aubert 75 76 1969, Fitton et al. 1988, Gauld & Dubois 2006, Korenko 2017, Korenko et al. 2015, Matsumoto & Takasuka 2010, Vincent 1979). Spiders of the family Theridiidae are 77 78 thought to successfully avoid predation by spider-hunting wasps of the families Sphecidae and Crabronidae by means of their 3D web architectures, based on the low frequency of 79 80 predation records of theridiids in comparison with that of 2D orb weaving spiders 81 (Blackledge et al. 2003), excluding Chalybion (Hymenoptera: Sphecidae) species, which exceptionally prefer theridiids, in addition to other spiders (e.g. Landes et al. 1987, Muma 82 & Jeffers 1945, Ward 1969). The adoption of theridiids as hosts by Zatypota spp. has 83 involved the successful evolution of specific offensive behaviours to overcome the 84 85 intricate defensive 3D web architectures.

*Zatypota maculata* Matsumoto & Takasuka 2010, on which we focus, exclusively parasitises the theridiid spider, *Nihonhimea japonica* (Bösenberg & Strand 1906) (Matsumoto & Takasuka 2010), which was recently transferred from *Parasteatoda* by Yoshida (2016). All three species of the genus *Nihonhimea* construct a characteristic web called a "knockdown 3D web", which consists of a non-viscid intricate 3D cobweb, a 91 retreat made of a dead leaf at the centre, and a dense non-viscid sheet web at the bottom 92 that serves as a capturing device (Figs 1, 4c, Benjamin & Zschokke 2003; Fig. 6C, Eberhard 1972, Eberhard et al. 2008; Fig. 42D-F, Jörger & Eberhard 2006; Figs 9, 19, 93 Yoshida 2003, Yoshida 2016; Figs 18-19). At the moment a prey insect is knocked down 94 95 by the cobweb and trapped by the sheet, the spider immediately drops down from the 96 retreat onto the underside of the sheet by penetrating the sheet, resulting in a small hole 97 on the sheet. It them bites and wraps the captured prey from underneath (cf. Takasuka 2013). 98

The knockdown 3D web seems to be particularly effective in avoiding predation by 99 100 spider-hunting aculeates because N. *japonica* has been scarcely recorded as stored prev 101 of sphecid (Sceliphrini), crabronid (Trypoxylini), or pompilid wasps. There is only a 102 record of N. japonica stored by Pison (Pison) punctifrons Shuckard, 1838 (Crabronidae), but just one individual among 74 individuals from four families, Theridiidae (mainly 103 104 Parasteatoda tepidariorum (C. L. Koch, 1841)), Araneidae, Tetragnathidae, and Salticidae (Iwata 1964). However, Z. maculata has successfully evolved offensive 105 oviposition behaviours specifically to cope with the knockdown 3D web of N. japonica. 106 107 In the present study, we report observations on these unique offensive oviposition behaviours of Z. maculata both in situ and in vitro, with the establishment of an 108 109 observational system in vitro.

110

## 111 Materials and methods

## 112 **Observation of oviposition behaviour** *in situ*

We conducted field observations in two periods and locations in Japan, Satsukiyama,
Ikeda city, Osaka Prefecture, in 2006 by RM, and the campus of Kobe University, Kobe

115 City, Hyogo Prefecture in 2015 by KT; however, there were no observations on 116 oviposition success at the latter. Instead, the campus played a role in being a resource of 117 spiders and adult wasps for the oviposition experiments in vitro. The observed spiders constructed their webs on planted Osmanthus fragrans Lour. var. aurantiacus Makino 118 119 (Oleaceae) in Ikeda and on O. fortunei Carrière (Fig. 1a) in the Kobe University campus. 120 A survey of the spider host population on 4 September 2015 on the campus resulted in 49 parasitised spiders (44 eggs, four young and one old instar larvae) out of 62 individuals, 121 122 and four cocoons inside empty retreats, resulting in 80.3% (53/66) parasitism. Almost all spiders bore egg sac(s) or hatched spiderlings inside their retreats. Sequences of female 123 124 wasps' behaviour in Ikeda were recorded using a digital single lens reflex camera, Nikon 125 D100 (Nikon Corporation), by RM.

126

### 127 Observation of oviposition behaviour in vitro

128 Several N. japonica were collected together with their own retreat (dead leaf) and 129 incorporated into an artificial substructure for web construction. It consisted of two 130 corrugated fibreboards cut in a doughnut-shape above and below, connected by wooden sticks (Fig. 1b). We hung the substructure with a knockdown 3D web and its owner inside 131 a large plastic reservoir ( $60 \text{ cm} \times 43 \text{ cm} \times 30 \text{ cm}$ ) laid sideways. After closing the reservoir 132 with a fine mesh cloth, three adult female Z. maculata wasps (individuals A, B and C) 133 134 collected on the campus were introduced into the reservoir one at a time. They were fed with a sugary isotonic drink (Aquarius, manufactured by Coca-Cola Japan), which 135 achieved maintenance of another polysphinctine (Z. albicoxa) for a long time (Takasuka 136 & Matsumoto 2011b, Takasuka et al. 2009). The wasp was replaced with another 137 individual when she died, and spiders that had not been attacked were re-used in the 138

succeeding experiment. We recorded the wasps' oviposition behaviour with a digital
single lens camera DMC-GH1 (Panasonic Corporation) fixed on a tripod. We judged an
attack as successful by the spider's paralysis caused by the wasp's sting.

In addition to normal oviposition, we qualitatively tested infanticidal behaviour. In other polysphinctines, it is known that previously attached immature wasps (previous occupants) on a spider host are removed by a subsequent ovipositing female wasp with rubbing movements of her ovipositor (Eberhard 2000, Gonzaga & Sobczak 2007, Takasuka & Matsumoto 2011a). We provided a parasitised spider, which remarkably bore both a middle instar larva and an egg (i.e. it was superparasitised) once each to individuals B and C, i.e. the same individual spider was used twice.

149

## 150 **Results**

#### 151 **Oviposition behaviour** *in situ*

152 A female was witnessed for the entire sequence of oviposition. In the first phase, the wasp 153 stayed on a leaf below the sheet, to which one of the supporting structures was attached. The wasp flew up to cling to one of the upper outermost supporting structures (Fig. 2a) 154 155 and crept around along it. Then, she flew again, landed on the lower supporting structure 156 and slowly climbed up and down threads of the supporting structure and knock-down trap 157 (sheet), towards the retreat, facing the retreat (Fig. 2b). Once she reached the spider host's 158 retreat, the spider escaped for a short distance, approximately 25 mm away from the 159 retreat (Fig. 2c), before the wasp made contact. The wasp remained motionless with her fore legs raised beside (Fig. 2c) and approximately 5 minutes later, on the spider's return 160 161 to the retreat, she pounced and quickly stung it, causing temporary paralysis. She grabbed the spider's abdomen and legs with her fore and middle legs (Fig. 2d), and gave several 162

additional stings into the bases of the coxae. Subsequently, she repeatedly rubbed her ovipositor over the anterior and lateral faces of the host abdomen. Then, an egg was laid on the anterolateral face of the host abdomen. It took approximately 15 minutes and 30 seconds for the wasp to reach the retreat and the duration between sting and oviposition was 260 seconds. Approximately 15 minutes later, the spider recovered from paralysis and hid herself inside the retreat listlessly.

In an incomplete observation on another individual, in which the wasp was already inside the supporting structures above the sheet and rather close to the retreat, the wasp similarly crept toward the retreat and then entered it. She succeeded in paralysing and ovipositing on the host without it escaping.

173

#### 174 Oviposition behaviour in vitro

In the oviposition experiment, five instances of successful oviposition (zero by wasp A, four by B, and one by C) were observed and two types of behaviour to gain access to spiders were recognised: 1) creeping up on a retreat as observed *in situ*; 2) diving into a sheet to lure a spider away from a retreat. Oviposition was observed on 5, 8, 11, and 15 September 2015 by wasp B and on 12 September 2015 by wasp C.

The typical "creeping-style" was observed in individual B (Supplementary video 1). We released the wasp from an empty vial to the lower fibreboard of the web substructure. She jumped 1 cm upwards, lay on her dorsum on the rear of the sheet, and then pulled herself through the sheet. Walking on the sheet to an edge of it, she climbed up the 3D cobweb to the part of the web above the retreat in which the spider and its spiderlings hid and climbed up and down repeatedly for approximately 24 minutes, gradually creeping up on the retreat, with several periods of motionlessness. Whilst the 187 wasp moved around the web, the spider stayed still in the retreat, with no observed 188 reaction. The wasp finally stopped 1 cm away from the retreat for 230 seconds before 189 eventually entering the retreat, resulting in the spider being stung and paralysed, and also 190 the spillage of several spiderlings. When the spider was paralysed, she grasped the 191 spider's abdomen by her fore and mid legs and rubbed it with her ovipositor and tip of 192 metasoma repeatedly at short intervals. Stopping metasoma movement, she laid an egg 193 onto the anterolateral face of the spider's abdomen. The duration between sting and 194 oviposition was 202 seconds. After oviposition, she left the retreat, rested for 58 minutes 195 30 seconds on the outer side of the retreat and finally flew away from the web. The spider 196 first moved again nine minutes after oviposition and a strange behaviour was observed. 197 As soon as the spider had recovered movement, it suddenly bit and wrapped up one of its 198 spiderlings and left it immediately outside the retreat. The spider re-entered the retreat without eating it. 199

200 Another three successes by wasp B were accomplished by means of the typical 201 "diving-style" (Supplementary video 2). She directly dived into a sheet from the air, the wooden stick or the lower fibreboard of the web substructure. Immediately after the wasp 202 203 dived into the web, the spider dropped or climbed down from the retreat and approached 204 her as potential prey along the underside of the sheet. At the moment of contact, the wasp 205 stung and paralysed the spider. In one case, the spider approached her but was very 206 cautious, returning to the retreat twice while the wasp feigned struggling on the sheet the entire time. At the third approach of the spider to the supposed prey, she pounced upon 207 the approaching spider from 1 cm away. In all three cases, the rubbing behaviour was 208 observed before oviposition, as described above. The duration between sting and 209 oviposition was 220, 225, and 245 (including ovicide) seconds, respectively. 210

211 Behavioural plasticity during a bout of oviposition by wasp C (Supplementary 212 video 3) was observed. After she dived into the sheet from right above the web through a 213 hole in the upper fibreboard ("diving-style"), but the spider, which obviously reacted to her diving, did not approach her. Being in a stalemate for 150 seconds, the wasp climbed 214 215 up the 3D cobweb and the spider subsequently climbed down as if she was potential prey, 216 resulting in the spider being stung. This appears to be a "fall-back method of luring" by 217 aggressively climbing following the failure in elicitation of the desired response from the spider by diving. The rubbing behaviour was observed and the duration between sting and 218 219 oviposition was 251 seconds (including ovicide). In this case, she was clearly observed 220 to pull her metasoma inwards and expelled an egg from the proximal end of the ovipositor (Fig. 3), i.e. neither from the ovipositor tip nor the tip of the metasoma (the genital 221 222 opening). This oviposition stance is called "ventral-press" sensu Takasuka et al. (2018), an adaptation to ovipositing on spiders' abdomens and preserved well within one of two 223 224 clades of polysphinctines (the other clade exhibits the reverse movement for laying on a 225 spider's cephalothorax). This is the fifth record of the ventral-press in this clade.

Wasp A was not observed to successfully attack a spider, but some attempts to gain access to the spider were observed. She frequently perched on the mid-height of the cobweb (supporting structures) and sometimes subsequently dropped onto and walked on the sheet. In one case, she crept up on the retreat but the spider dropped off it and stayed motionless on the periphery of the sheet. She entered the empty retreat, stayed there for four minutes, and was finally reconciled to flying away.

232

233 Ovicide

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When superparasitised spiders, bearing both a middle instar wasp larva and an egg,

235 were provided to wasps B and C, the same results occurred. After 15-30 seconds of 236 rubbing behaviour after the first sting, they immediately detected the egg and levered it 237 off with the ovipositor tip, removing the egg in merely 10-20 seconds (Supplementary videos 2, 1:07-1:30; 3, 1:05-1:17). The removed egg attached to the ovipositor was 238 239 eventually discarded by wiping her hind leg (Supplementary videos 2, 1:48; 3, 1:17). She 240 focused the rubbing behaviour exclusively on the left side of the spider's abdomen where the egg was attached and made additional stings into a membrane between the left coxae 241 (Supplementary videos 2, 2:15-2:23). She finally laid an egg onto the left side of the 242 abdomen dorso-laterally and flew away without removing the existing larva on the right 243 side of the abdomen, which was right in front of her. Thus, she was superparasitising the 244 spider again. We examined one of the removed eggs under the microscope and observed 245 246 embryonic movement inside the egg chorion, suggesting that the egg was not killed by 247 the removal. Although the additional sting observed in both cases with ovicide was not 248 detected in ovipositions upon unparasitised spiders, they were not clearly recorded in the 249 videos due to the position of the spider and the resolution of the videos.

250

## 251 **Discussion**

## 252 Oviposition tactics of *Z. maculata* with comparative ethology

We discovered behaviours of *Z. maculata* that are presumably species-specific, being specialised to the knockdown 3D web of *N. japonica. Zatypota maculata* females clearly recognised the sheet web, interpretable from their avoidance (creeping-style) and exploitation (diving-style) of the web. This is the third observation of oviposition behaviour of polysphinctines against theridiid spiders (all by *Zatypota*) and there are both unique and mutual characteristics among ours and the other two records. The comparison is schematised in an ethogram (Fig. 4).

260 Zatypota petronae Gauld, 1991 utilises Theridion evexum Keyserling, 1884 found 261 in Costa Rica (Barrantes & Weng 2007). The web of T. evexum includes a folded live leaf 262 that forms a conical retreat, with a tangle in front of the retreat opening, and long viscid 263 lines extending from the tangle to other leaves (Fig. 4a, Eberhard et al. 2008; Fig. 20A-264 B, Weng & Barrantes 2007). A female Z. petronae hovered in front of the retreat opening 265 and then flew inside it, resulting in a struggle between the wasp and spider hanging on a dragline (Weng & Barrantes 2007). The spider was finally paralysed, although the authors 266 did not confirm whether an egg was eventually laid. 267

268 Zatypota albicoxa (Walker, 1874) utilises Parasteatoda tepidariorum and several other congeners across the Palaearctic region (Bordoni 2003, Fitton et al. 1988, Iwata 269 270 1942, Matsumoto & Takasuka 2010, Nielsen 1923). The web of P. tepidariorum consists of a central retreat (generally made of silk thread tangles, but rarely of a dead leaf or 271 272 detritus) and numerous gum-footed vertical lines to suspend prey in the air (Fig. 4b, 273 Benjamin & Zschokke 2003; Figs. 6A, 8A, Griswold et al. 1998; Fig 2A-B, Takasuka & Matsumoto 2011b; Fig. 1). Females of Z. albicoxa have evolved several tactics for coping 274 275 with the gum-footed cobweb to gain access to P. tepidariorum (Fig. 4b): 1) by perching 276 on and hanging from one of the gum-footed lines to lure the spider (ambush-style; 277 Takasuka 2009b, Takasuka et al. 2009); 2) by perching on the mid-height on the cobweb 278 (non-sticky area) to lure the spider or to pounce directly on the spider when it is passing by (alternative ambush-style; Takasuka 2009a, Takasuka et al. 2009); 3) by climbing up 279 a non-sticky frame thread from the ground to gain access to the spider directly (climbing-280 style; Takasuka et al. 2009); and 4) by wandering under the web and laying on her dorsum 281 on the floor pulling at a gum-footed line with her fore/mid legs to lure the spider 282

283 (reclining-style; Takasuka 2011a, b, c, d, Takasuka & Matsumoto 2011b).

The two types of tactics employed by Z. maculata observed in this study (creeping 284 to gain access and the diving to imitate prey, Fig 4c) seem to demonstrate phenotypic 285 plasticity within an individual because both behaviours were practised by two individuals, 286 287 A and B (C showed plasticity by diving and climbing as a fall-back method). The 288 deployment of creeping-up tactics in both Z. maculata and Z. albicoxa (Takasuka et al. 2009) possibly indicates that this tactic is effective and potentially widely shared in the 289 genus Zatypota against theridiid 3D cobwebs, whose threads are mostly non-viscid. The 290 imitating tactic (aggressive mimicry), adapted to various types of theridiid cobwebs 291 292 would thus evolve differently in each host-parasitoid interaction. However, offensive behaviour of other members of *Zatypota* (the most species-rich polysphinctine genus) 293 remains to be discovered. 294

Offensive behaviours of Zatypota indicate that to subjugate spiders Zatypota wasps 295 296 adopt careful ways to approach so that the spiders do not escape from the wasp, rather 297 than avoiding becoming prey to the spiders, by means of an abrupt plunge (Z. petronae), 298 creeping-up (Z. albicoxa and Z. maculata), and aggressive mimicry, i.e. imitation of prey 299 and ambush (Z. albicoxa and Z. maculata) (Fig. 4, Table 1). Indeed, all failures in 300 oviposition in this study resulted from spiders escaping as a reaction to the proximity of 301 the wasp. Stealth or luring of the spider has also been observed in other genera of polysphinctines known for their offensive oviposition behaviour (Table 1), including a 302 plunge by Hymenoepimecis argyraphaga Gauld, 2000 and H. veranii Loffredo & 303 Penteado-Dias, 2009, aggressive mimicry by Brachyzapus nikkoensis (Uchida, 1928), 304 and pure ambush (i.e. no imitation) by Polysphincta sp. nr. purcelli Gauld, 1991 and P. 305 janzeni Gauld, 1991 (Eberhard 2000, Gonzaga & Sobczak 2007, Iwata 1942, Kloss et al. 306

#### 307 2016, Matsumoto 2009).

308 These conservative tactics among polysphinctines contrast with spider-hunting aculeates; many species of the family Pompilidae (Eberhard 1970, Preston-Mafham 1991, 309 310 Shimizu 1989), Sceliphron (Sphecidae, Sceliphrinae) (Blackledge & Pickett 2000, 311 Eberhard 1970), and Miscophus (Crabronidae, Miscophini) (Coville 1986), are known to 312 aggressively charge the web or spiders directly to flush and chase the spider. However, these groups seldom prey on Theridiidae. On the other hand, Chalybion seems to have 313 314 evolved aggressive mimicry against spiders of both Araneidae (Blackledge & Pickett 2000, Coville 1976) and Theridiidae (Uma & Weiss 2012). Chalybion californicum 315 316 (Saussure, 1867) climbs the cobweb to gain access to the prey spider, Parasteatoda tepidariorum (see the web sketch in Fig. 4b), as well as following the dropping spider, or 317 318 touches and vibrates gum-footed vertical line(s) from the bottom of the web to entice the spider (Uma & Weiss 2012), as with Z. albicoxa (Fig. 4b). These records suggest that 319 320 efficiently utilising Theridiidae involved the evolution of specific modes of offensive 321 behaviour to cope with the complex 3D web architecture, such as creeping-up and aggressive mimicry, as seen in both Chalybion and Zatypota. However, only Z. maculata 322 has efficiently coped with the knockdown 3D web by means of a diving-style of 323 324 aggressive imitation of prey.

325

## 326 Ovicide by Z. maculata

327 Several polysphinctines are known to remove previous occupants on a spider host 328 (infanticide) when encountering an already parasitised spider. In all cases, this was 329 accomplished by means of rubbing movements of the ovipositor (Eberhard 2000, 330 Gonzaga & Sobczak 2007, Takasuka & Matsumoto 2011a). Infanticide by removal would

331 have evolved de novo among polysphinctines because the immediate outgroup of the 332 group is comprised of parasitoids of egg-sacs or egg-nests of spiders (Matsumoto 2016), 333 which seem unable to remove previous occupants enclosed by an egg-sac or egg-nest. In a quantitative study of polysphinctine infanticide by Takasuka and Matsumoto (2011a), 334 335 ovipositing Z. albicoxa always removed any previous occupants regardless of the time 336 costs or instar of the previous occupant because only one larva can complete its 337 development even when superparasitism occurs (the opponent will be discarded immature with the spider carcass). This determination to commit infanticide results in 338 superparasitism being very rare in this species; superparasitism was not observed among 339 340 201 immature Z. albicoxa individuals attached to spiders from egg to penultimate instar larva (Takasuka & Tanaka 2013). 341

Although it was only two trials, two *Z. maculata* also committed ovicide through the removal of an existing egg, as does *Z. albicoxa*, whereas both failed to detect the superparasitising larva. This indicates that once infant/ovicide is successfully accomplished, the behaviour is not repeated (although subsequent rubbing behaviour was observed), because in the field ovipositing females seldom encounter doubly parasitised spiders. However, this hypothesis needs to be quantitatively tested.

348

## 349 Acknowledgments

We express our cordial thanks to Gavin Broad (Natural History Museum, London) for his critical reading of the manuscript. This work was partly supported by JSPS KAKENHI Grant Number JP16K18620, JSPS Research Fellowships for Young Scientists No. JP17J40065 to KT and by JSPS KAKENHI Grant Number 23770099 to RM.

| 354 | Legends of figures   |
|-----|--|
| 355 | Figure 1. Knockdown 3D web of Nihonhimea japonica. (a) The web constructed in situ         |
| 356 | (the campus of Kobe University) on Osmanthus fortunei. (b) The web reconstructed on a      |
| 357 | web substructure.  |
| 358 |  |
| 359 | Figure 2. Sequence of offensive oviposition behaviour of Zatypota maculata in situ. (a)    |
| 360 | Clinging to one of the upper outermost supporting structures. (b) Creeping up on and       |
| 361 | facing the retreat in which the spider host hides. (c) Wasp and spider in a stalemate. (d) |
| 362 | Grabbing the paralysed spider's abdomen (different individual).                            |
| 363 |  |
| 364 | Figure 3. Zatypota maculata lays an egg upon the abdomen of Nihonhimea japonica by         |
| 365 | means of the ventral-press sensu Takasuka et al. (2018).                                   |
| 366 |  |
| 367 | Figure 4. Schemata of host theridiid webs utilised by three polysphinctines and ethogram   |
| 368 | of offensive oviposition behaviours of polysphinctines. (a) Zatypota petronae against      |
| 369 | Theridion evexum (Weng & Barrantes 2007). (b) Zatypota albicoxa against Parasteatoda       |
| 370 | tepidariorum (Takasuka et al. 2009, Takasuka & Matsumoto 2011b). (c) Zatypota              |
| 371 | maculata against Nihonhimea japonica.  |
| 372 |  |
| 373 | Supplementary video 1. The typical creeping-style exhibited by wasp B. See the text for    |
| 374 | detailed explanation. The video was partly sped up by 30 times.                            |
| 375 |  |
| 376 | Supplementary video 2. The typical diving-style with ovicide upon a previous egg (1:07-    |
| 377 | 1:30) exhibited by wasp B. See the text for detailed explanation.                          |
| 378 |  |
| 379 | Supplementary video 3. The diving-style (failure) and then the fall-back method of         |
| 380 | luring by aggressively climbing (success) with ovicide upon a previous egg (1:05-1:17)     |
| 381 | exhibited by wasp C. See the text for detailed explanation. The video was partly sped up   |
| 382 | by 30 times.   |
| 383 |  |

| species   | spider host   | web type  | oviposition style   | tactics classification  | reference   |
|---|---|---|---|---|---|
| Zatypota<br>maculata  | <i>Nihonhimea japonica</i> (Theridiidae)                            | knockdown 3D<br>web   | <ol> <li>creeping</li> <li>diving into sheet</li> </ol>   | creeping-up<br>aggressive mimicry   | this study  |
| Zatypota<br>petronae  | Theridionevexum(Theridiidae)  | 3D web with<br>long viscid lines<br>(Fig. 4a)                     | intruding into a retreat  | abrupt plunge   | Weng & Barrantes (2007)   |
| Zatypota<br>albicoxa  | Parasteatoda<br>tepidariorum<br>(Theridiidae)                       | 3D web with<br>gum-footed<br>vertical lines<br>(Fig. 4b)          | <ol> <li>ambush (hanging<br/>from a vertical line)</li> <li>alternative ambush<br/>(perching at a mid-<br/>height of the web)</li> <li>climbing</li> <li>reclining against a<br/>vertical line</li> </ol> | aggressive mimicry<br>aggressive mimicry<br>creeping-up<br>aggressive mimicry | Takasuka <i>et al.</i><br>(2009), Takasuka &<br>Matsumoto (2011b) |
| Hymenoepimecis<br>argyraphaga                               | Leucauge argyra<br>(Walckenaer, 1841)<br>(Tetragnathidae)           | horizontal orb<br>web   | plunging from above   | abrupt plunge   | Eberhard (2000)   |
| Hymenoepimecis<br>veranii                                   | Araneusomnicolor(Keyserling,1893)(Araneidae)                        | vertical orb web<br>with a detached<br>retreat and<br>barrier web | plunging from barrier<br>thread   | abrupt plunge   | Gonzaga &<br>Sobczak (2007)                                       |
| Brachyzapus<br>nikkoensis                                   | Agelena silvatica Oliger,<br>1983 (Agelenidae)                      | funnel sheet web  | diving into sheet   | aggressive mimicry  | Iwata (1942),<br>Matsumoto (2009)                                 |
| Polysphincta sp.<br>nr. purcelli<br>Polysphincta<br>janzeni | Cyclosa fililineata<br>Hingston, 1932 and C.<br>morretes Levi, 1999 | vertical orb web  | intentionally flushing a<br>spider and then<br>ambushing the<br>returning spider  | pure ambush   | Kloss <i>et al.</i> (2016)  |

**Table 1** Known styles of offensive behaviour by polysphinctines and classification of their tactics

#### 386 **References**

- Aubert, J. F. (1969) Les Ichneumonides ouest-paléarctiques et leurs hôtes. 1. Pimplinae,
   Xoridinae, Acaenitinae. Paris.
- Barrantes, G. & J. L. Weng (2007) Natural history, courtship, feeding behaviour and
   parasites of *Theridion evexum* (Araneae: Theridiidae). *Arachnology*, 14, 61-65.
- Benjamin, S. P. & S. Zschokke (2003) Webs of theridiid spiders: construction, structure
  and evolution. *Biol J Linn Soc*, 78, 293-305.
- Blackledge, T. A., J. A. Coddington & R. G. Gillespie (2003) Are three-dimensional
  spider webs defensive adaptations? *Ecol Lett*, 6, 13-18.
- Blackledge, T. A. & K. M. Pickett (2000) Predatory interactions between mud-dauber
  wasps (Hymenoptera, Sphecidae) and *Argiope* (Araneae, Araneidae) in captivity. *J Arachnol*, 28, 211-216.
- Bordoni, A. (2003) Osservazioni su Zatypota albicoxa (Walker) (Hymenoptera,
  Ichneumonidae) e sul suo ospite Achaearanea lunata (Clerck) (Araneae,
  Theridiidae). Doriana, 8, 1-4.
- 401 Coville, R. E. (1976) Predatory behavior of the spider wasp, *Chalybion californicum*402 (Hymenoptera, Sphecidae). *Pan-Pac Entomol*, **52**, 229-233.
- 403 Coville, R. E. (1986) Spider-Hunting Sphecid Wasps. In: *Ecophysiology of Spiders*: 309404 318. W. Nentwig (Ed.). Springer-Verlag, Berlin, Germany.
- 405 Eberhard, W. G. (1970) The predatory behavior of two wasps, Agenoideus humilis

#### 406 (Pompilidae) and Sceliphron caementarium (Sphecidae), on the orb weaving

- 407 spider Araneus cornutus (Araneidae). Psyche: A Journal of Entomology, 77, 243408 251.
- 409 Eberhard, W. G. (1972) Observations on the biology of Achaearanea tesselata (Araneae:

Theridiidae). Psyche: A Journal of Entomology, 79, 209-212.

| 411 | Eberhard, W. G. (2000) The natural history and behavior of Hymenoepimecis            |
|-----|--|
| 412 | argyraphaga (Hymenoptera: Ichneumonidae) a parasitoid of Plesiometa argyra           |
| 413 | (Araneae: Tetragnathidae). J Hymenoptera Res, 9, 220-240.                            |
| 414 | Eberhard, W. G., I. Agnarsson & H. W. Levi (2008) Web forms and the phylogeny of     |
| 415 | theridiid spiders (Araneae: Theridiidae): chaos from order. Syst Biodivers, 6, 415-  |
| 416 | 475.   |
| 417 | Fitton, M. G., M. R. Shaw & I. D. Gauld (1988) Pimpline ichneumon-flies Hymenoptera, |
| 418 | Ichneumonidae (Pimplinae). London, U.K.: Royal Entomological Sciety of               |
| 419 | London.  |
| 420 | Gauld, I. D. & J. Dubois (2006) Phylogeny of the Polysphincta group of genera        |
| 421 | (Hymenoptera : Ichneumonidae; Pimplinae): a taxonomic revision of spider             |
| 422 | ectoparasitoids. Syst Entomol, 31, 529-564.  |
| 423 | Gómez Durán, J. M. & C. van Achterberg (2011) Oviposition behaviour of four ant      |
| 424 | parasitoids (Hymenoptera, Braconidae, Euphorinae, Neoneurini and                     |
| 425 | Ichneumonidae, Hybrizontinae), with the description of three new European            |
| 426 | species. ZooKeys, 125, 59-106.   |
| 427 | Gonzaga, M. O. & J. F. Sobczak (2007) Parasitoid-induced mortality of Araneus        |
| 428 | omnicolor (Araneae, Araneidae) by Hymenoepimecis sp. (Hymenoptera,                   |
| 429 | Ichneumonidae) in southeastern Brazil. Naturwissenschaften, 94, 223-227.             |
| 430 | Greeny, H. F., L. A. Dyer & A. M. Smilanich (2012) Feeding by lepidopteran larvae is |
| 431 | dangerous: A review of caterpillars' chemical, physiological, morphological, and     |
| 432 | behavioral defenses against natural enemies. Invertebrate Survival Journal, 9, 7-    |

433

34.

| 435 | orb-web building spiders (Araneae, Orbiculariae: Deinopoidea, Araneoidea). Zool         |
|-----|---|
| 436 | J Linn Soc, <b>123</b> , 1-99.  |
| 437 | Gross, P. (1993) Insect behavioral and morphological defenses against parasitoids. Annu |
| 438 | <i>Rev Entomol</i> , <b>38</b> , 251-273.   |
| 439 | Iwata, K. (1942) Biology of some Japanese Polysphincta. Mushi, 14, 98-102.              |
| 440 | Iwata, K. (1964) Ethological notes on four Japanese species of Pison (Hym. Sphecidae).  |
| 441 | Mushi, <b>38,</b> 1-6.  |
| 442 | Jörger, K. M. & W. G. Eberhard (2006) Web construction and modification by              |
| 443 | Achaearanea tesselata (Araneae, Theridiidae). J Arachnol, 34, 511-523.                  |
| 444 | Kloss, T. G., M. O. Gonzaga, J. A. M. Roxinol & C. F. Sperber (2016) Attack behavior of |
| 445 | two wasp species of the Polysphincta genus group (Hymenoptera,                          |
| 446 | Ichneumonidae) on their orb-weaver spider hosts (Araneae, Araneidae). J Insect          |
| 447 | Behav, <b>29</b> , 315-324.   |
| 448 | Korenko, S. (2017) First record from Italy of Zatypota anomala (Ichneumonidae,          |
| 449 | Ephialtini), a parasitoid of the cribellate spider Dictyna pusilla (Araneae,            |
| 450 | Dictynidae). Arachnol Mitt, 54, 1-4.  |
| 451 | Korenko, S., J. Satrapová & K. Zwakhals (2015) Manipulation of araneid spider web       |
| 452 | architecture by the polysphinctine parasitoid Zatypota picticollis (Hymenoptera:        |
| 453 | Ichneumonidae: Pimplinae). Entomol Sci, 18, 383-388.                                    |
| 454 | Landes, D. A., M. S. Obin, A. B. Cady & J. H. Hunt (1987) Seasonal and latitudinal      |
| 455 | variation in spider prey of the mud dauber Chalybion californicum (Hymenoptera,         |
| 456 | Sphecidae). J Arachnol, 15, 249-256.  |
| 457 | Matsumoto, R. (2009) "Veils" against predators: modified web structure of a host spider |

Griswold, C. E., J. A. Coddington, G. Hormiga & N. Scharff (1998) Phylogeny of the

- 458 induced by an ichneumonid parasitoid, *Brachyzapus nikkoensis* (Uchida)
  459 (Hymenoptera). *J Insect Behav*, 22, 39-48.
- 460 Matsumoto, R. (2016) Molecular phylogeny and systematics of the *Polysphincta* group
  461 of genera (Hymenoptera, Ichneumonidae, Pimplinae). *Syst Entomol*, 41, 854-864.

Matsumoto, R. & K. Takasuka (2010) A revision of the genus Zatypota Förster of Japan,

- 463 with descriptions of nine new species and notes on their hosts (Hymenoptera:
- 464 Ichneumonidae: Pimplinae). *Zootaxa*, **2522**, 1-43.

- 465 Muma, M. H. & W. F. Jeffers (1945) Studies of the spider prey of several mud-dauber
  466 wasps. *Ann Entomol Soc Am*, 38, 245-255.
- 467 Murphy, J. A. & M. J. Roberts (2015) *Spider Families of the World and Their Spinnerets*.
  468 York: British Arachnological Society.
- 469 Nielsen, E. (1923) Contributions to the life history of the pimpline spider parasites
  470 (*Polysphincta, Zaglyptus, Tromatobia*) (Hym. Ichneum.). *Entomol Medd*, 14, 137471 205.
- 472 Preston-Mafham, R. (1991) Spiders: An Illustrated Guide. London, U.K.: Quatro
  473 Publishing plc.
- 474 Shimizu, A. (1989) An ethological study of *Agenioideus ishikawai* (Hymenoptera,
  475 Pompilidae). *Jpn J Entomol*, **57**, 654-662.
- 476 Takasuka, K. (2009a) Zatypota albicoxa attacking her host Achaearanea tepidariorum
  477 under the artificial condition. Movie Archives of Animal Behavior Data No.:
  478 momo090727za02a. Online at <u>http://www.momo-p.com/showdetail-</u>
- 479 <u>e.php?movieid=momo090727za02a</u>
- 480 Takasuka, K. (2009b) *Zatypota albicoxa* showing ambush style to entice and attack her
  481 host, *Achaearanea tepidariorum. Movie Archives of Animal Behavior* Data No.:

482 momo090727za01a. Online at <u>http://www.momo-p.com/showdetail-</u>
483 e.php?movieid=momo090727za01a

Takasuka, K. (2011a) Zatypota albicoxa attacking her host, Parasteatoda tepidariorum,
by means of the reclining style. Movie Archives of Animal Behavior Data No.:
momo100416za02a. Online at <a href="http://www.momo-p.com/showdetail-e.php?movieid=momo100416za02a">http://www.momo-p.com/showdetail-</a>

Takasuka, K. (2011b) *Zatypota albicoxa* showing reclining style to entice and attack her
 host, *Parasteatoda tepidariorum*. *Movie Archives of Animal Behavior* Data No.:
 momo100415za01a. Online at <a href="http://www.momo-p.com/showdetail-">http://www.momo-p.com/showdetail-</a>

491 <u>e.php?movieid=momo100415za01a</u>

Takasuka, K. (2011c) Zatypota albicoxa showing reclining style to entice and attack her
 host, Parasteatoda tepidariorum 2. Movie Archives of Animal Behavior Data No.:
 momo100416za01a. Online at <a href="http://www.momo-p.com/showdetail-e.php?movieid=momo100416za01a">http://www.momo-p.com/showdetail-</a>
 e.php?movieid=momo100416za01a

Takasuka, K. (2011d) Zatypota albicoxa wandering under the web of her host, *Parasteatoda tepidariorum*, to detect the vertical thread. Movie Archives of
Animal Behavior Data No.: momo100416za03a. Online at <a href="http://www.momo-p.com/showdetail-e.php?movieid=momo100416za03a">http://www.momo-p.com/showdetail-e.php?movieid=momo100416za03a</a>

Takasuka, K. (2013) Predatory behavior by *Nihonhimea japonica* by means of
 knockdown 3D web. *Movie Archives of Animal Behavior* Data No.:
 momo131018pj01b. Online at <a href="http://www.momo-p.com/showdetail-e.php?movieid=momo131018pj01b">http://www.momo-p.com/showdetail-</a>

Takasuka, K., N. R. Fritzén, Y. Tanaka, R. Matsumoto, K. Maeto & M. R. Shaw (2018)
 The changing use of the ovipositor in host shifts by ichneumonid ectoparasitoids

| 506 | of spiders (Hymenoptera, Ichneumonidae, Pimplinae). Parasite, 25, 17.                   |
|-----|---|
| 507 | Takasuka, K. & R. Matsumoto (2011a) Infanticide by a solitary koinobiont ichneumonid    |
| 508 | ectoparasitoid of spiders. Naturwissenschaften, 98, 529-536.                            |
| 509 | Takasuka, K. & R. Matsumoto (2011b) Lying on the dorsum: unique host-attacking          |
| 510 | behaviour of Zatypota albicoxa (Hymenoptera, Ichneumonidae). J Ethol, 29, 203-          |
| 511 | 207.  |
| 512 | Takasuka, K., R. Matsumoto & N. Ohbayashi (2009) Oviposition behavior of Zatypota       |
| 513 | albicoxa (Hymenoptera, Ichneumonidae), an ectoparasitoid of Achaearanea                 |
| 514 | tepidariorum (Araneae, Theridiidae). Entomol Sci, 12, 232-237.                          |
| 515 | Takasuka, K. & K. Tanaka (2013) Seasonal life cycle of Zatypota albicoxa (Hymenoptera:  |
| 516 | Ichneumonidae), an ectoparasitoid of Parasteatoda tepidariorum (Araneae:                |
| 517 | Theridiidae), in southwestern Japan. Pac Sci, 67, 105-111.                              |
| 518 | Uma, D. B. & M. R. Weiss (2012) Flee or fight: ontogenetic changes in the behavior of   |
| 519 | cobweb spiders in encounters with spider-hunting wasps. Environ Entomol, 41,            |
| 520 | 1474-1480.  |
| 521 | Vincent, L. S. (1979) A new record for Sinarachna anomala (Hymenoptera:                 |
| 522 | Ichneumonidae), an external parasitoid of Mallos pallidus (Araneae: Dictynidae).        |
| 523 | Pan-Pac Entomol, 55, 192-194.   |
| 524 | Ward, G. L. (1969) The occurrence of Chalybion zimmermanni Dahlbom (Sphecidae) in       |
| 525 | Indiana. Proc Indiana Acad Sci, 79, 231-233.  |
| 526 | Weng, J. L. & G. Barrantes (2007) Natural history and larval behavior of the parasitoid |
| 527 | Zatypota petronae (Hymenoptera : Ichneumonidae). J Hymenoptera Res, 16, 326-            |
| 528 | 335.  |
| 529 | Yoshida, H. (2003) The Spider Family Theridiidae (Arachnida: Araneae) from Japan [in    |

530

Japanese]. Tokyo, Japan: Arachnological Society of Japan.

- Yoshida, H. (2016) *Parasteatoda, Campanicola, Cryptachaea* and two new genera
  (Araneae: Theridiidae) from Japan. *Bulletin of the Yamagata Prefectural Museum*,
  34, 13-30.
- Yu, D. S., K. van Achterberg & K. Horstmann, (2016) Taxapad 2016, World
  Ichneumonoidea 2015. Database on flash-drive. Ottawa, Ontario, Canada.

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Figure 3 Takasuka et al.

# (a) *Zatypota petronae* against *Theridion evexum* (Weng & Barrantes 2007)



(b) Zatypota albicoxa against Parasteatoda tepidariorum (Takasuka et al. 2009, Takasuka & Matsumoto 2011b)



fall-back method of luring

Figure 4 Takasuka et al.

living plant(s)

creeping-up

for the returning flushed spider

(creeping-style)

## **Supporting Information**

Additional Supporting Information may be found in the online version of this article:

**Video S1.** The typical creeping-style exhibited by wasp B. See the text for detailed explanation. The video was partly sped upby 30 times.

https://zslpublications.onlinelibrary.wiley.com/action/downloadSupplement?doi=10.111 1%2Fjzo.12668&file=jzo12668-sup-0001-VideoS1.mp4

**Video S2.** The typical diving-style with ovicide upon a previous egg (1:07–1:30) exhibited by wasp B. See the text for detailed explanation.

https://zslpublications.onlinelibrary.wiley.com/action/downloadSupplement?doi=10.111 1%2Fjzo.12668&file=jzo12668-sup-0002-VideoS2.mp4

**Video S3.** The diving-style (failure) and then the fall-back method of luring by aggressively climbing (success) with ovicide upon a previous egg (1:05-1:17) exhibited by wasp C. See the text for detailed explanation. The video was partly sped up by 30 times.

https://zslpublications.onlinelibrary.wiley.com/action/downloadSupplement?doi=10.111 1%2Fjzo.12668&file=jzo12668-sup-0003-VideoS3.mp4