

Tetrastichus brachyopae (Hymenoptera: Eulophidae) new to the Netherlands, reared from *Brachyopa* larvae (Diptera: Syrphidae)

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Tetrastichus brachyopae is reported as new to the Netherlands from De Kaaistoep, a nature reserve close to the city of Tilburg (province of Noord-Brabant) and not far from the Belgium border. This eulophid wasp is exclusively known as natural enemy of hoverflies of the genus *Brachyopa*. The hoverfly larvae develop in sap runs, mostly of various deciduous tree species. The wasps were bred from the puparia which were obtained as larvae from an oak. Both *B. bicolor* and *B. insensilis* were found as hosts. *Tetrastichus brachyopae* is a gregarious koinobiont endoparasitoid, with clutch sizes varying from 7-18 individuals per host. The emergence sex ratios show a strong female bias, indicating local mate competition, where brothers compete over a limited number of mates, increasing parental fitness if more daughters are produced.

Introduction

Saproxilic hoverflies of the genus *Brachyopa* reproduce in sap runs or sappy wounds, mostly found on different kinds of deciduous trees. Much of the life and behaviour of the larvae, developing as bacterial filter feeders, needs to be discovered. From the nineteen European species of *Brachyopa*, eight were found in The Netherlands (Reemer et al. 2010). Unlike many other syrphids, *Brachyopa* species do not show mimicry with wasps or bees, but rather resemble house flies (Muscidae) or dung flies (Scatophagidae) (figure 1). At our study site *Brachyopa bicolor* (Fallén) was the dominant species, but *B. insensilis* Collin was also present in a sap run of a common oak (*Quercus robur*). Limited information is available as under which ecological conditions different *Brachyopa* species are able to coexist. *Brachyopa bicolor* is largely associated with mature beech and goat moth (*Cossus cossus* (Linnaeus)) infested common oak, whilst *B. insensilis* prefers elms and horse chestnut (Ball & Morris 2000, Rotheray 1993, Sjuts 2004). Reason to write this article was the occurrence of the hymenopterous parasitoid *Tetrastichus brachyopae* Graham (Hymenoptera: Eulophidae: Tetrastichinae), which we here report as a new species of the Dutch fauna. *Tetrastichus brachyopae* is currently considered to be a natural enemy of a larger number of European *Brachyopa* species (www.bioinfo.org.uk/html/Tetrastichus_brachyopae.htm). The parasitoid wasp likely strongly decreases the survival of the aggregated hoverfly larvae in sap runs.

Tetrastichus brachyopae was treated by Graham (1991) as a member of the *Tetrastichus murcia* group in the highly diverse and species rich subfamily Tetrastichinae. The European species of this group are endoparasitoids of three families of brachycerid

Diptera: Stratiomyidae, Xylomyidae and *Brachyopa* (Syrphidae). Members of the genus *Tetrastichus* however have a wider range of hosts, such as Chrysomelidae, Coccinellidae, Curculionidae and Scolytidae (Coleoptera), Cecidomyiidae and Chloropidae (Diptera), and Argidae and Tenthredinidae (Hymenoptera) (Graham 1991). Although *Brachyopa* is not phylogenetically related to Stratiomyidae and Xylomyidae, the larvae show some similar modifications, like being rather hard-bodied and slow and stiff in their movements, dorso-ventrally depression, and narrower headed than the thorax. It may be that shape, cuticula function and behaviour make them attractive to the same group of parasitoids. Remarkably, other syrphids must be able to cope with very different taxa of parasitoids (Noyes 2016, Rotheray 1993). In this article we describe the habitat of the host, present the results of a breeding experiment, and evaluate taxonomic characters to identify *T. brachyopae*. Some aspects of the ecology in relation to the hosts are elucidated.

Site description

De Kaaistoep is a nature reserve in the province of Noord-Brabant, close to the city of Tilburg. The direct surrounding of the collecting locality consists of nutrient poor sandy soil with mixed forest of less than fifty years old. The forest mainly consists of common oak and northern red oaks (*Q. rubra*), birch (*Betula*), Scots pine (*Pinus sylvestris*) and fir (*Picea*), with undergrowth of rowan (*Sorbus aucuparia*), *Rhododendron*, black cherry (*Prunus serotina*) and a few herbaceous plants.

The first author inspected a single free standing common



1. Male *Brachyopa insensilis*. Photo: S. Bot
1. Mannetje *Brachyopa insensilis*.



2. The oak from which *Brachyopa insensilis* and *Tetrastichus brachyopae* were collected. The sap run is clearly visible. 22 March 2010. Photo: A. van Eck
2. De eik waarop *Brachyopa insensilis* en *Tetrastichus brachyopae* zijn verzameld. De sapstroom is duidelijk zichtbaar. 22 maart 2010.

oak with an estimated diameter of 45 cm (at 1,5 meter) in a forested garden near a sandy path (figure 2). The locality is sheltered and quite shady in summer.

Sampling and breeding methods

On 29 May 2009, *Brachyopa insensilis* was collected at a sap run of common oak (figure 2). Almost one year later, on 22 March 2010, 45 *Brachyopa* larvae were collected from the same tree in order to rear them. They were kept in vials, stored at room temperature, with some sap (resins and fluids) in which they were found in the tree. Vials were checked regularly for humidity, and to see if puparia were present. If puparia were found, they were transferred using a pair of tweezers, to small petri dishes, with one puparium in each dish.

The tree was inspected again on 15 March 2011. Because much lower numbers of larvae were present (compared to 2010) only a few were sampled and kept in a vial as described until pupation. The number of collected larvae was not recorded this time. No other adult flies of *Brachyopa* have been collected from this tree after 2009.

Results

Many of the approximately 45 larvae found in 2010 did not pupate and no *Brachyopa* adult was bred successfully (table 1). Only four larvae pupated and these turned out to be parasitised. From the larvae collected in 2011, one *Brachyopa bicolor* was reared and two puparia were parasitised.

The hatched parasitoids all belong to the species of *Tetrastichus brachyopae* (see below). This species is a gregarious endoparasitoid which attacks *Brachyopa* larvae. It was already present in the collected host larvae. The clutch size varies from seven to eighteen per puparium. The calculated emergence sex ratio shows a strong female bias (Chi-squared test, $P < 0.001$) and the number of male specimens is very low; in one of our rearing cases only females emerged (table 1). However, in the latter case examination of the puparium showed one adult male wasp which did not hatch from the puparium, and two dead pupae.

The wasps emerged from the puparia through a single tiny opening. Immediately after emergence they started to mate. Males were restless and immediately mated after a female emerged. Duration of copulation lasted less than one second and within a short time all females were inseminated. Each female seemed to become unattractive to a male after mating.



3. Male *Tetrastichus brachyopae*. Photo: A. Polaszek
3. Mannetje *Tetrastichus brachyopae*.



4. Female *Tetrastichus brachyopae*. Photo: A. Polaszek
4. Vrouwtje *Tetrastichus brachyopae*.

Identification of hosts and parasitoid

Material examined: 13 females and 3 males of *T. brachyopae*. Specimens from the puparia which pupated 26 April 2010 and emerged 9 June 2010 (table 1) were deposited in the entomological collection of The Natural History Museum, London (BMNH). All other specimens remain in the collection of the first author.

Hosts *Brachyopa* adults were identified with keys in Thompson (1980) and Doczkal & Dzioczek (2004) and the most recent descriptions in Reemer *et al.* (2009). One parasitised puparium was

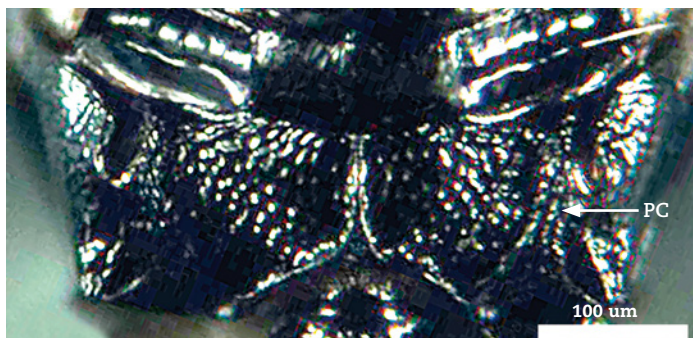
identified as *B. insensilis* by using the keys in Krivosheina (2005) and Rotheray (1993), three puparia were identified as *B. bicolor* and two puparia could not with certainty be identified due to damage (table 1).

Parasitoid Large genera such as *Tetrastichus* may be problematic to identify, because of their small size and incomplete descriptions in the literature. Knowledge of the host(s) and host habitat(s) is often of great importance. The description of *Tetrastichus brachyopae* by Graham is based on specimens reared from an unknown *Brachyopa* species in April 1960 in Moravia, eastern

Table 1. Overview of reared *Brachyopa spec.* and *Tetrastichus brachyopae*. The dead male *T. brachyopae* is left out.

Tabel 1. Overzicht van uitgekweekte *Brachyopa spec.* en *Tetrastichus brachyopae*. Het dode mannetje *T. brachyopae* is weggelaten.

Sampling date of larvae / verzameldatum van larven	Date of pupation / datum van verpopping	Date of emergence / datum van verschijning	Species / soort	Reared		Sex-ratio / seksratio (= ♂/(♂+♀))	
				<i>Brachyopa</i> / gekweekte <i>Brachyopa</i>	<i>Tetrastichus brachyopae</i> / gekweekte <i>Tetrastichus brachyopae</i>		
				♂	♀		
22.iii.2010	5.iv.2010	2.vi.2010	<i>B. bicolor</i>		1	7	0.13
22.iii.2010	26.iv.2010	8.vi.2010	<i>B. spec.</i>		1	6	0.14
22.iii.2010	29.iv.2010	9.vi.2010	<i>B. cf bicolor</i>		4	14	0.22
22.iii.2010	29.iv.2010	10.vi.2010	<i>B. bicolor</i>		2	10	0.17
15.iii.2011	18.iii.2011	5.iv.2011	<i>B. bicolor</i>	<i>B. bicolor</i> 1 ♂			
15.iii.2011	18.iii.2011	30.iv.2011	<i>B. bicolor</i>		2	14	0.13
15.iii.2011	unknown	4.v.2011	<i>B. insensilis</i>		0	8	0.00



5. Propodeum of *Tetrastichus brachyopae*. PC = paraspiracular carina (arrow). Photo: A. Polaszek
5. Propodeum van *Tetrastichus brachyopae*. PC = paraspiraculaire carina (pijl).



6. Antenna of male *Tetrastichus brachyopae*, with nine segments. Scapula black (brown in picture due to light exposure), laterally flattened. Photo: A. Polaszek
6. Antenne van man *Tetrastichus brachyopae*, met negen segmenten. Zwarte, zijdelings afgeplatte scapula (bruin op de foto door belichting).

Czech Republic (Graham 1991). To identify *T. brachyopae* we used a set of characters given in the original description following the morphological terminology of Graham (1987, 1991). Both sexes (figures 3-4): antenna with scape and pedicel without raised sculpture; scutellum with two pairs of setae; malar sulcus present, extending from eye to mouth edge; fore wing postmarginal vein 0.5× or less the length of stigmal vein; dorsellum undivided; propodeum (figure 5) with plicae extending from hind margin to spiracle. Paraspiracular carina present (PC in figure 5), extending to hind corner of propodeum. Frons with median longitudinal carina extending from the toruli no more than halfway to median ocellus; mouth width much narrower than malar space; antennal scape slightly shorter than maximum eye length. Female: ovipositor sheaths protruding slightly beyond tip of gaster, which is ovate, not elongate. Antenna (figure 7): clava about the same length as F2+F3, with no construction between C1 and C2. submedian lines of scutellum equidistant from each other and from sublateral lines.

Male Antenna as in figure 6, other non sex-specific characters as in female.

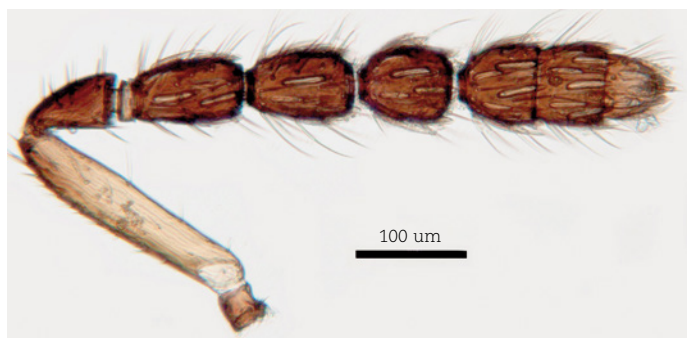
Distribution of *Tetrastichus brachyopae*

The presence of *T. brachyopae* was previously confirmed for four countries: Sweden (Hedqvist 2003), Germany (Vidal 1997) reared from *Brachyopa pilosa* Collin, Czech Republic (Graham 1991), and Switzerland (single specimen in BMNH, Hedqvist 2003). According to Alexander (2002) it was also found in Britain in 1993, but specimens to confirm this are not present either in the BMNH collection or in the collection of NBC-Naturalis, Leiden, The Netherlands.

Discussion

Today, three species of *Brachyopa* are confirmed hosts for *T. brachyopae*: *B. pilosa* (Noyes 2016, Vidal 1997), *B. bicolor* (this publication) and *B. insensilis* (this publication).

From the breeding results we conclude that *T. brachyopae* is already present in the larval stages of the host. The final developmental steps to pupation take place in the puparia. *Tetrastichus brachyopae* presumably oviposits in younger larval stages in late spring, and hibernates inside the host. Further development takes place in the next year, when the hoverfly larva will grow and reach maturity. This koinobiont life style of the parasitoid fits in the life cycle of most *Brachyopa*, which is supposed to be univoltine. Reemer *et al.* (2009) present unimodal fly histograms, with almost all *Brachyopa* species peaking in May. However, *Brachyopa* larvae of other species and of different sizes



7. Antenna of female *Tetrastichus brachyopae*, with eight segments. Scapula yellow and slender. Photo: A. Polaszek
7. Antenne van vrouw *Tetrastichus brachyopae*, met acht segmenten. Scapula geel en slank.

and developmental stages can be found at the same time at the same spot, suggesting that some of the fly larvae may also be facultatively bivoltine (Rotheray 1993, this study). If so, the parasitoid must also be able to hibernate twice. Further study is required to test this hypothesis.

We can think of no straightforward explanation for the extremely high larval mortality of the hoverflies. In 2010, only four out of 45 larvae reached the pupal stage. Perhaps the relative humidity indoors became too low, resulting in the death of bacteria in the sap, thus depriving the larvae of their main food source. The winter of 2009/10 was cold (www.knmi.nl), and larvae were collected one week later than the next year. Pupation took place approximately one month after collecting. In the winter of 2010-2011, on the contrary, only December was very cold, but January and February were mild and early spring was extremely dry, sunny and warm (www.knmi.nl). Pupation already took place a few days after collecting of the larvae. Obviously, weather conditions prior to the collecting must have been of influence on the survival of the larvae on the tree and it may have had influence on the survival and the emergence dates as well, even when bred indoors.

Observations on the mating behaviour of *T. brachyopae* show that in a short time all sisters were inseminated by their brother(s). The populations of many gregarious chalcidoid wasp species are structured in such a way that mating is not random and occurs between members of local patches. When patches are founded by a single wasp, and all mating occurs between siblings, brothers may compete for mating. This local mate competition (LMC) selects for a female-biased sex ratio, especially in species where females have control over offspring sex,

as in the parasitic Hymenoptera with a haplodiploid sex determination mechanism (Beukeboom & Perrin 2014, Godfray 1994, Quicke 1997). In fact, the production of one son (no competition) is enough to inseminate all daughters. *Tetrastichus brachyopae* clearly belongs to a very large group of Hymenoptera within this reproduction model. Theoretically, two factors are predicted to decrease the degree of female bias: (1) an increase in the

number of foundress females in the patch and (2) an increase in the fraction of individuals mating after dispersal from the natal patch. In case of *T. brachyopae*, males are winged and may disperse between patches to find virgins, for instance unmated females from an all-female brood. Mothers often produce some more than only one son per brood (table 1).

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Samenvatting

***Tetrastichus brachyopae* (Hymenoptera: Eulophidae) nieuw voor Nederland, gekweekt uit *Brachyopa*-larven (Diptera: Syrphidae)**

Tetrastichus brachyopae is voor het eerst in Nederland aangetroffen in De Kaaistoep, een natuurreservaat nabij Tilburg (Noord-Brabant), niet ver van de grens met België. Dit eulophide bronswespje is specifiek bekend als natuurlijke vijand van zweefvliegen van het genus *Brachyopa*. De larven van deze zweefvliegen ontwikkelen zich in sapstromen, vooral van diverse soorten loofbomen. De bronswespjes werden opgekweekt uit puparia van sapzweefvliegen die eerder als larven waren verzameld van een zomereik. De vrij algemeen voorkomende *Brachyopa bicolor* (gedeuhte sapzweefvlieg) en de zeldzamere *B. insensilis* (kale sapzweefvlieg) werden beide vastgesteld als gastheren. *Tetrastichus brachyopae* blijkt een gregaire, koinobionte endoparasitoid te zijn, met 7 tot 18 nakomelingen per pop. De geslachtsverhouding van de uitgekomen wespjes is erg scheef, want er worden vooral vrouwtjes geproduceerd. Dit impliceert local mate competition (LMC), een voortplantingsstrategie waarbij de fitness van ouders toeneemt bij de productie van dochters als de broers slechts hoeven te concurreren om een beperkt aantal partners. Het typerende paringsgedrag van het bronswespje wordt summier beschreven.



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