Nectar plants and larval food-plants of the genus Glossotrophia (Geometridae, Sterrhinae): studies on pollen grains attached to museum specimens

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Abstract. 544 specimens of the Geometrid moth genus *Glossotrophia*, belonging to nine species, were examined under the stereomicroscope searching for attached pollen grains. Pollen was found on 105 specimens (8 species). In addition we identified pollen grains of further 39 specimens under the scanning electron microscope. Pollen grains attached to the tip of the abdomina of nine females strongly indicate egg deposition at or in those flowers (mainly Caryophyllaceae). Pollen grains attached to the proboscis or neighbouring structures, most frequently found in the subgenus *Glossotrophia*, and here preferably in females, are suggestive of nectar plant use. The subgenus *Glossotrophia* turned out to be almost exclusively specialised on Caryophyllaceae nectar plants (mainly *Silene, Dianthus*), whereas the other subgenera utilize a wider spectrum of nectar sources. This finding does well correspond to the known spectra of larval host-plants. A strong positive correlation was found between length of proboscis, preference for Caryophyllaceae, and frequency of pollen grain attachment. Collectively, these results demonstrate that the analysis of pollen loads in museum specimens (though sometimes being very old) may provide valuable data on host use patterns through time and space.

Key words. Geometridae, Sterrhinae, *Glossotrophia*, pollen load, museum specimens, nectar sources, host plants, Caryophyllaceae

Introduction

Pollen loads have previously been studied in different groups of animals, such as mammals (Goldingay et al. 1991; Hacket & Goldingay 2001), birds (Hopper 1980; Wooller et al. 1983; Borgella et al. 2001), bees (Bernhardt & Walker 1984; Westrich & Schmidt 1987; Camillo & Garofalo 1989; Abrol 1990; Nazarov 1995; Müller 1996; Nazarov & Gerlach 1997), flies (Krannitz & Maun 1991; Kearns 1992), butterflies (Cruden & Hermann-Parker 1979; Hawkeswood 1985; Tobar et al. 2001) and moths (Esche 1992; Gregg 1993; Nazarov & Efetov 1993). This is an indirect method to get information on nectar-plant relationships of the studied specimens. Pollen load can be used as a marker for migration (Gregg 1993) or for determination of the extent of host-plant specialisation (Westrich & Schmidt 1987; Müller 1996). Further it was used to determine the role of specific animals as pollinators of particular plant species (Hopper 1980; Goldingay et al. 1991; Hacket & Goldingay 2001) and to discover food sources and food niche overlap among sympatric species (Camillo & Garofalo 1989; Esche 1992). We here study pollen loads of the taxonomically difficult genus Glossotrophia. Identification and systematics of this genus were subject of various previous papers (Hausmann 1992, 1993a, b, 1994, 1996). Some taxa of this genus have a very long proboscis. The length of the proboscis is about 10–13mm in subgenus *Glossotrophia*, 7-10mm in subgenus Libanonia, 4-6mm in subgenus Parenzanella, and only 3-4mm in subgenus Microglossotrophia (Hausmann 1993b).

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The long proboscis of the nominotypical subgenus *Glossotrophia* is unique in Geometridae and inspired Prout (1913) to base the scientific name on this feature. The curved proboscis externally projects from the pupa and is much longer than the whole pupa (Hausmann 2001, text fig. 113). As until now nothing is known about nectar plant interactions of the adult moths and data on larval host-plant relationships are scarce (Tab. 2), we aimed to analyse pollen loads attached to different body parts of the different sexes and taxa of *Glossotrophia* in order to obtain more information on food-plants. Using museum specimens, we show that analysis of pollen loads in museum specimens (though sometimes being very old) may provide valuable indicators of host use patterns through time and space.

Material and Methods

Light microscopy. 544 specimens of the collection material of the genus *Glossotrophia* in the Zoologische Staatssammlung München (ZSM) (Tab. 2) were examined under a stereomicroscope in order to determine a statistical pattern about the frequency of pollen loads in the genus *Glossotrophia*. In a standardised way we examined each specimen for 30sec. All details, such as label data, sex, position of pollen grains and if possible pollen type were noted (special labels were attached to specimens with pollen grains). Reared specimens were excluded from the study. If long series were available, representative random samples from selected localities were examined. Real frequency of pollen grain attachment is underestimated, since (1) some pollen grains have surely been overlooked given the limited time of examination, (2) doubtful observations of grains were scored as negative findings, (3) during collecting usually the more beautiful and thus younger specimens are chosen, which may not had visited flowers, (4) possibly some reared specimens, that are not labelled as such, were included in the study and (5) some specimens could have lost the pollen grains after collecting.

SEM studies: 39 further specimens where pollen was visible under the stereomicroscope were chosen for detailed examination and identification of the pollen grains (these specimens are not included in the frequency analysis). All pollen grains were studied if there were just a few; otherwise we examined a random sample. The airdried pollen grains from the moths were taken up on double faced adhesive tape, sputtered with gold and studied under a scanning electron microscope (Philips XL 30 ESEM) at 20 kV. We used the works of Punt (1976), Punt et al. (1981), Punt & Clarke (1980, 1981, 1984), and Punt & Hoen (1995) to identify pollen grains.

Statistical analysis: To analyse the data we employed a Chi²-test (Rohlf and Sokal 1995; Sokal and Rohlf 1995). If the expected value for at least one cell was equal to or smaller than 5 we carried out Fisher's exact test instead.

Results

We studied under the SEM more than 250 pollen grains attached to 39 specimens belonging to 10 *Glossotrophia* taxa (6 species; Tab. 1). More than 60% of these grains could be identified as Caryophyllaceae pollen. The subgenus *Glossotrophia* exhibited

the highest specialisation to plants of this plant family: 5 of the 6 examined taxa had exclusively Caryophyllaceae pollen grains, mostly from *Silene* or *Dianthus* (Figs. 2a, b). The only exception was *Glossotrophia diffinaria* where, in addition to *Silene* pollen, grains from *Linum catharticum* (Fig. 2c), cf. *Polygonum* and an unknown pollen grain were found. In contrast, examination of taxa from the subgenera *Libanonia* and *Parenzanella* revealed pollen types from a diverse spectrum of plant families. On one female (specimen Nr. 14b, Tab. 1) we found Caryophyllaceae pollen grains on the last segment of the abdomen.

105 specimens with pollen grains were found among 544 examined specimens studied under the stereomicroscope (Tab. 3). Though it was not possible to identify the plant species or families under the stereomicroscope, most of the pollen grains (>70%) seemed to correspond well to the Caryophyllaceae pollen grains as examined under the SEM.

We found pollen in all subgenera and on most studied taxa (Tab. 3). Especially in the subgenus *Glossotrophia* (Chi²_{df=1}=6.36; p=0.012) pollen occurred more frequently on females than on males. No pollen grains could be found on males of the subgenus *Microglossotrophia*. In the remaining three subgenera, 6–21% of the males carried pollen grains (difference between these three subgenera not significant: Chi²_{df=2}=3.35; p=0.19). In females there were big differences with regard to pollen load at subgeneric level (Chi²_{df=3}=19.8; p<0.001). Females of the subgenus *Glossotrophia* had an exceptionally high load (pollen grains on 35% of the studied specimens).

In all subgenera most pollen was found attached to head parts (Tab. 4). 86–100% of the pollen-positive specimens in the different subgenera had pollen grains at least on one of the head parts. When looking only at the head, the most important structure for pollen load in all subgenera, except for *Parenzanella*, was the proboscis. In the subgenus *Glossotrophia*, for example, we found 70% of the 'head-positive' specimens carrying pollen on the proboscis, in *Parenzanella* only 38% (Chi²_{df=1}=6.76; p<0.01). In the latter subgenus, the most important structure of the head for pollen load was the eye.

Comparing the sexes with respect to pollen attachment to different body parts there are barely differences with one exception: In 15% of the cases pollen could be found on the tip of abdomen on females, but pollen grains were never attached to the tip of abdomen on males (Fig. 1).

Discussion

Most species of the *Silene vulgaris* and *S. dioica*-groups are considered 'good' nectar plants for nocturnal moths. They produce a lot of nectar in the late afternoon or evening until midnight (Witt et al. 1999). Pollen grains attached to eyes, palpi, frons or proboscis of various *Glossotrophia* species may be interpreted as strong indication of nectaring at *Silene* species, as does the frequent presence of pollen grains at the underside of wings (mainly at the basis). In 105 of 544 examined specimens (19%) pollen grains were found, almost all of them attached to the mouthparts or



Fig. 1: Sex-specific attachment of pollen grains to various body parts of *Glossotrophia* moths in relation to specimens with pollen grains (results from stereo microscope examination). Bars with the same letters are not significantly different (p < 0.05). Comparisons were made only within single body parts (*: Fisher's exact test).

close to them, and most of the pollen grains apparently belonged to the family Caryophyllaceae. Thus pollen grains attached to collection specimens revealed that Caryophyllaceae are important as food source for Geometrid moths of the genus *Glossotrophia*. The authors have no explication for the extraordinarily high frequency of pollen grains at the eyes of *G. asellaria* (subgenus *Parenzanella*), though pollen frequency was low at the proboscis of this species (Tab. 4).

The following conclusions are drawn from our studies:

(1) Species of the subgenus *Glossotrophia* seem to feed nearly exclusively on nectar from Caryophyllaceae, mainly *Silene* and *Dianthus*. Only the Turkish *G. diffinaria* was recorded also on Linaceae, and probably Polygonaceae. One attached Orchidaceae pollinarium was observed in *G. confinaria prouti* (Fig. 2d). The other subgenera had a much wider spectrum of nectar-plants (Tab. 1).

(2) Overall females bear pollen grains more often than males. This difference was pronounced in the subgenus *Glossotrophia*, but absent in *Libanonia, Parenzanella* and *Microglossotrophia*. Our findings might indicate that females visit flowers more often than males. Since in other moths adult feeding affects the size or number of off-spring produced by a female (e.g. Boggs 1987; Stevens et al. 2002), also *Glossotrophia* females might be in greater need of carbohydrate intake.

(3) Attachment of pollen grains at the last segment(s) of female abdomen (as found in 9 females) may be interpreted as strong indication of egg deposition into the calyx

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Fig. 2: Pollen found at different *Glossotrophia* taxa. (a) Caryophyllaceae pollen attached to head parts of *Glossotrophia confinaria*, (b) Pollen grain of the *Silene dioica*-group, (c) Pollen grain of *Linum catharticum*, (d) Pollinium of *Platanthera* (Orchidaceae) attached to head of *Glossotrophia confinaria prouti* (northern Italy).

of the flower. No males were found with pollen grains at the tip of the abdomen. In one female of *Glossotrophia confinaria prouti* (specimen Nr. 14, Tab. 1) eight Caryophyllaceae pollen grains were identified under the SEM. Caryophyllaceae as larval host-plants are already known for this species (Tab. 2). Similarly pollen grains at the tip of female abdomen have been observed under the stereo microscope in nine further females belonging to *G. confinaria*, *G. diffinaria*, *G. alba*, *G. mentzeri* and *G. rufomixtaria* (all subgenus *Glossotrophia*). Pollen grains could be identified in additional studies under the SEM as 'Caryophyllaceae'.

(4) Known larval host-plants for the subgenus *Glossotrophia* are largely restricted to the Caryophyllaceae genus *Silene*. The Spanish *G. rufomixtaria* was recorded also on *Gypsophila* and, reputedly, on *Dianthus* (references in Tab. 2). Thus, larval host-plants do well correspond to the preferred adult nectar sources (Tab. 2) in this subgenus. This may indicate a narrow niche of the adult moths, i.e. nectaring, mating and oviposition would all occur close to host-plants. In the monobasic subgenus *Parenzanella* (including only *G. (P) asellaria*), however, larval host-plants of many other families (Zygophyllaceae, Scrophulariaceae, Lamiaceae; Brassicaceae) are recorded (Hausmann in press). Similarly, a much wider spectrum of nectar sources was observed than in the subgenus *Glossotrophia*.

(5) Length of proboscis is about 10–13mm in subgenus *Glossotrophia*, 7–10mm in subgenus *Libanonia*, 4–6mm in subgenus *Parenzanella*, and only 3–4mm in sub-

genus *Microglossotrophia* (Hausmann 1993b). Thus, length of proboscis reveals to be strongly correlated to frequency of pollen grain attachment to proboscis and palpi (Tabs. 2, 4). While this frequency is 21.6% in *Glossotrophia*, the subgenus with the longest proboscis, it is only 1.6% in *Microglossotrophia*, the subgenus with the shortest proboscis, and about 6% in *Libanonia* and *Parenzanella*, the subgenera with medium sized probosci. These differences are significant ($\text{Chi}^2_{df=3}=33.3$; p<0.001). Taking into consideration the results discussed under (1), correlation results also between length of proboscis and Caryophyllaceae as nectar source. Analyzing the SEM results, Caryophyllaceae pollen was found on 25 of in total 28 studied specimens of *Glossotrophia*, the subgenus with the longest proboscis, while Caryophyllaceae pollen was only found on 4 of in total 11 studied specimens of subgenera with shorter probosci (difference significant in Fisher's exact test; p=0.002).

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Tab. 1. Examined taxa, number of observed pollen grains, body parts, where grains were attached to, and identification of pollen types using a SEM.

Specimen Nr.	Sex	Number of pollen grains	Pollen grains attached to	Pollen type
Glossotroph	ia (Glo	ssotrophia) confi	inaria confinaria	
1	f	2	leg	Silene vulgaris-type
2	m	3	proboscis, palpi, eye	Dianthus
3	m	3	wing	Silene vulgaris-type
4	m	1	proboscis	Silene vulgaris-type
5	m	4	proboscis	Silene dioica-group
6	f	4	eye	Caryophyllaceae
7	m	4	wing	Caryophyllaceae
8	m	9	proboscis, palpi	Silene dioica-group
9	m	1	proboscis	Silene dioica-group
Glossotroph	ia (Glo	ssotrophia) confi	inaria prouti	
10	f	6	proboscis, leg	Dianthus
11	f	10	, proboscis, eye	4 x Silene dioica-group, 6 x Dianthus
12	m	22	proboscis	Dianthus
13	m	2	proboscis	Caryophyllaceae
14a	f	11	proboscis	Dianthus
14b	f	8	tip of abdomen	Caryophyllaceae
Glossotroph	ia (Glo	ssotrophia) confi	inaria scoblei	
15	m	6	wing	Caryophyllaceae
Glossotroph	ia (Glo	ssotrophia) diffir	iaria	
16	f	3 .	proboscis	1 x cf. Polygonum, 1 x Silene dioica group, 1 x Silene vulgaris-type
17	f	10	proboscis, palpi	9 x Silene vulgaris-type, 1 x unidentified (not Caryophyllaceae)
18	f	4	proboscis	Linum catharticum
19	f	9	proboscis	Linum catharticum
20	f	6	proboscis, wing	Linum catharticum
Glassatroph	- ia (Gle	ssotronhia) alba	alha	
21	f f	6	proboscis	Caryophyllaceae
22	f	3	proboscis, eye	Silene dioica-group
23	f	2	wing	Silene vulgaris-type
24	f	2	proboscis	Silene vulgaris-type
Glossotroph	ia (Glo	ssotrophia) rufo	mixtaria	
25	m	6	proboscis	3 x Dianthus, 3 x Caryophyllaceae
26	f	2	proboscis, eye	Silene dioica-group
27	m	1	proboscis	Silene dioica-group
28	m	8	proboscis	Caryophyllaceae
Glossotroph	ia (Lib	anonia) semitata	taurica	
29	f	5	proboscis	4 x Caryophyllaceae, 1 x Linum catharticum
30	f	12	proboscis	cf. Polygonum
Glossotroph	ia (Lib	anonia) semitata	ariana	
31	f	7	proboscis	cf. Polemonium
32	f	13	proboscis	Syringa
33	m	4	proboscis	Caryophyllaceae
34	f	10	proboscis	Silene dioica-group
35	m	14	proboscis	12 x cf. <i>Polemonium</i> , 2 x unknown (not Caryophyllaceae)
Glossotroph	ia (Pa	renzanella) asella	aria isabellaria	
36	f	13	proboscis	cf. Polemonium
37	f	10	proboscis	cf. Polemonium
Glossotroph	ia (Par	renzanella) asella	aria dentatolineata	
38	f	1	proboscis	Caryophyllaceae
39	f	7	wing	Syringa

Tab. 2. Studied material with results from the analysis of pollen loads; * selection of specimens randomly, focussing on 2-3 regions, but omitting selection of reared specimens; ** apparently no Caryophyllaceae pollen

larval food plant families	Caryophyllaceae ⁵	Caryophyllaceac ^{1,6}		,	,	Caryophyllaceae ²	Caryophyllaccae ¹	Caryophyllaceae ^{1,2,5}	,	,		Scrophulariaceae 2.5	Brassicaceae ^{2.4}			Zygophyllaccac ²	Zygophyllaccac, Scrophulariaccac, Lamiaccac ^{2,3}		I	
nectar plant families	Caryophyllaceae ¹	Caryophyllaccac, Orchidaceae	Caryophyllaccae	Caryophyllaceac, Linaceae,	?Polygonaceae			Caryophyllaceae	Caryophyllaceac, Linaceae,	Polemoniaceae, Cleaceae, Carvonhvillaceae	car y oprigramate	ı	? Polemoniaceae 1	Oleaceae, Caryophyllaceae						
length of proboscis (mm)	10-13	10-13	10-13	10-13	10-13	10-13	10-13	10-13	7-10	7-10	4-6	46	46	4-6	4-6	4-6	4-6	3-4	34	
specimens with attached pollen: altitude (m above sea- level)	200-800	300-400	700-1200	600-1700	100-1600		550-1100	0-1000	400-1000	1100-1800	400-800	0-100	0-1100	600-2000	1000	,	0-300	700		ohlfahrt (1981
specimens with attached pollen: collecting date (months from 1-X11)	late V -early IX	late VI – early IX	mid-VI – late VII	mid-VI – early VIII	early VI - mid-VIII		mid-V - late VII	mid-VI - late VII; lat IX	mid-IV – late VI, early IX	early V – alte VI; early 1X – mid-X	early VI - mid-VI	early IV; mid-VIII – mid-IX	late IV - late V;	mid-VI - mid-VII	mid-IV; carly VII	ı	latc V - carly VI	early IV		1935); ⁶ : Forster & We
specimens with attached pollen in	6	5	-	5	4			4	7	Ś		1	2	7			ı.	,		: Prout (
specimens with attached pollen in binocular studies	20	7	7	20	×		S	7	6	1	4 **	4 **	9	7	3	ı	4	1		iċre (1871); ⁵
origin	SE France (Hautes Alpes), N Italy (Lake Garda), W Macedonia (Lake Ochrid)	N Italy, Val Venosta	N Sicily	Turkey	C Italy	S Italy	Crcte	S France, Spain	S Turkcy	E Aphganistan. Turkmenistan	Corsica, Sardinia	C Italy	N and E Spain	S Spain	Morocco	Canary islands	SE Algeria, Libya	SW Saudi Arabia; Yemen	Oman	(4); ³ : Prout (1935); ⁴ : Mill
number of randomly selected (*)specimens for stereo microscope studies	73	38	10	29	30	29	19	40	32	31	14	15	42	20	17	25	61	31	30	ausmann (200
total specimens present at ZSM (approx.)	400	200	10	60	40	200	30	09	001	300	14	15	80	25	20	25	30	200	500	ötterl); ² : Ha
Taxon	G. (Glossotrophia) confinaria confinaria (Herrich-Schäftör, 1847)	G. (Glossotrophia) confinaria prouti Hummann 1903	G. (Glossotrophia) confinaria scoblei	G. (Glossotrophia) diffinaria Prout. 1913	G. (Glossotrophia) alba alba Hausmann.	G. (Glossotrophia) alba brunellii Lamman 1003	G. (Glossotrophia) mentzeri Hausmann.	G. (Glossotrophia) rufomixtaria (de Graslin, 1863)	G. (Libanonia) semitata taurica Wehrli, 1930	G. (Libanonia) semitata ariana Ebert, 1965	G. (Parenzanella) asellaria asellaria (Herrich-Schäffer, 1847)	G. (Parenzanella) asellaria romanaria Miliòre 1869)	G. (Parenzanov) G. (Parenzanov) G. (Parenzanov)	(multer, 1000) G. (Parenzanella) asellaria dentatolineata Wehrli, 1926	G. (Parenzanella) asellaria lenzi Hausmann, 1903	G. (Parenzanella) asellaria gersthergeri Hausmann 1903	G. (Parenzanella) asellaria tripolitana Turati, 1929	G. (Microglossotrophia) alfierii Wiltshirc.	G. (Microglossotrophia) gracilis Brandt, 1941	1: results from present studics (Hausmann & D

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Tayon	total ex	xamined	with pollen grains
1 8 2011	males	females	males females
Glossotrophia (Glossotrophia) confinaria	41	32	8 (20%) 12 (38%)
confinaria			
Glossotrophia (Glossotrophia) confinaria prouti	22	16	2 (9%) 5 (31%)
Glossotrophia (Glossotrophia) confinaria scoblei	6	4	5 (83%) 2 (50%)
Glossotrophia (Glossotrophia) diffinaria	14	15	9 (64%) 11 (73%)
Glossotrophia (Glossotrophia) alba alba	15	15	1 (7%) 7 (47%)
Glossotrophia (Glossotrophia) alba brunellii	15	14	0 (0%) 0 (0%)
Glossotrophia (Glossotrophia) mentzeri	15	4	4 (27%) 1 (25%)
Glossotrophia (Glossotrophia) rufomixtaria	14	26	1 (7%) 6 (23%)
Glossotrophia (Libanonia) semitata taurica	16	16	2 (13%) 4 (25%)
Glossotrophia (Libanonia) semitata ariana	15	16	0 (0%) 1 (6%)
Glossotrophia (Parenzanella) asellaria asellaria	13	1	3 (23%) 1 (100%)
Glossotrophia (Parenzanella) asell'aria	10	5	3 (30%) 1 (20%)
romanaria			
Glossotrophia (Parenzanella) asellaria	27	15	4 (15%) 2 (13%)
isabellaria			
Glossotrophia (Parenzanella) asellaria	12	8	2 (17%) 0 (0%)
dentatolineata			
Glossotrophia (Parenzanella) asellaria lenzi	11	6	0 (0%) 3 (50%)
Glossotrophia (Parenzanella) asellaria	14	11	0 (0%) 0 (0%)
gerstbergeri			
Glossotrophia (Parenzanella) asellaria	9	10	2 (22%) 2 (20%)
tripolitana			
Glossotrophia (Microglossotrophia) alfierii	15	16	0 (0%) 1 (6%)
Glossotrophia (Microglossotrophia) gracilis	12	18	0 (0%) 0 (0%)
subgenus Glossotrophia (total)	142	126	30 (21%) 44 (35%)
subgenus Libanonia (total)	31	32	2 (6%) 5 (15%)
subgenus Parenzanella (total)	96	56	14 (15%) 9 (16%)
subgenus Microglossotrophia (total)	27	34	0 (0%) 1 (3%)
TOTAL	296	248	46 (16%) 59 (24%)

Tab. 3. Frequency of pollen grains attached to *Glossotrophia* specimens (selected taxa) after examination under a stereomicroscope.

Tab. 4. Attachment of pollen grains to various body parts of the moths (results from stereo microscope examination; compare Tab. 3); **ab**: absolute numbers (multiple entries possible); pp: frequency of occurrence in relation to specimens with pollen grains; **pt**: frequency of occurrence in relation to total number of examined specimens; **S**: total numbers of examined specimens; n: specimens with pollen grains.

subgenus	Glossotrophia			L	Libanonia			Parenzanella				Microglossotrophia				total		
body part	ab	pp	pt	ab	pp	pt		ab	pp	pt		ab	pp	pt	ab	pp	pt	
proboscis	45	0.61	0.17	4	0.57	0.06		8	0.35	0.05		1	1.00	0.02	58	0.55	0.11	
palpi	28	0.38	0.10	1	0.14	0.02		2	0.09	0.01		-	-	-	31	0.30	0.06	
eye	25	0.34	0.09	3	0.43	0.05		14	0.61	0.09		-	-	-	42	0.40	0.08	
frons	4	0.05	0.01	-	-	-		1	0.04	0.01		-	-	-	5	0.05	0.01	
antennae	5	0.07	0.02	-	-	-		1	0.04	0.01		-	-	-	6	0.06	0.01	
legs/thorax	15	0.20	0.06	-	-	-		1	0.04	0.01		-	-	-	16	0.15	0.03	
wings	28	0.38	0.10	3	0.43	0.05		4	0.17	0.03		-	-	-	35	0.33	0.06	
abdomen	11	0.15	0.04	-	-	-		2	0.09	0.01		-	-	-	13	0.12	0.02	
tip of abdomen	8	0.11	0.03	-	-	-		-	-	-		-	-	-	8	0.08	0.01	
n	74				7			23				1				105		
_	268				63		152				61				544			

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