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Article

Different breeding conditions affect the morphological variability in larvae of *Platytrombidium fasciatum* (Trombidiformes: Microtrombidiidae)

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Abstract

The impact of temperature and light/dark cycle on morphological traits of *Platytrombidium fasciatum* was examined in larvae obtained from field-collected females. The eggs laid by 65 females at laboratory conditions were assigned to four experimental groups. Varied thermal conditions affected the values of nine out of 46 (nonparametric MANOVA) and 26 out of 37 (LDA) morphometric traits in larvae. As many as six quantitative traits differed significantly irrespective of the method (MANOVA, LDA) applied. The analysis of the metric traits within the dorsal sclerites revealed that the lower mean temperature over the period of egg incubation, translated into longer duration of development, was associated with an increase in distance between the bases of 3rd pair of non-sensillary setae on scutum at the decrease in distance between the bases of 2nd pair of non-sensillary setae as well as in distance between the level of sensilla and the posterior edge of the sclerite. In the case of 40 measurable traits in larvae of *P. fasciatum*, the variability going beyond the hitherto knowledge on the species was observed.

Key words: Parasitengona, temperature, light/dark cycle, morphology, intraspecific variation

Introduction

Platytrombidium Thor, with *P. africanum* (Oudemans), *P. curtipilosum* (Schweizer), *P. fasciatum* (C.L. Koch), and *P. uencense* (Feider), is one of over 90 genera in the subfamily Microtrombidiinae (Mąkol & Wohltmann 2012, 2013). Among the genus members, only *P. fasciatum* has been known from all active life instars - larva, deutonymph, and adult (Mąkol & Wohltmann 2012).

Active postlarval forms of *P. fasciatum* can be easily distinguished from other, sympatrically and/or synoptically occurring species, by the presence of white, transverse stripes, and sometimes also white spots, against the bright red coloration of idiosoma dorsum. In general, the light spots or stripes, as an effect of depigmentation of particular groups of setae, have been relatively rarely observed in other members of Microtrombidiidae and related families of Trombidioidea of western, central and southern Europe. The presence of peculiar 'white pattern' has been known e.g. for *Gonothrombium oudemansianum* (Feider, 1948) (Microtrombidiidae) and *Neothrombium* sp. (Neothrombiidae) (Feider, 1948 and unpublished data).

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Larvae of *P. fasciatum*, obtained by experimental rearing, were studied by Robaux (1972). The most comprehensive redescription of the species, based on representatives of all active instars, and including data on biology was provided by Gabryś et al. (2005). Until present, the *P. fasciatum* remains the only member of *Platytrombidium* reported from Poland. It was recorded also from France, Spain, Germany, Romania, and Italy (Gabryś et al. 2005; Mąkol & Wohltmann 2012), whereas its presence in northern Europe (Fennoscandia) was questioned (Stålstedt et al. 2019). Larvae of *P. fasciatum* have been known to parasitize Diptera of the families Anthomyzidae, Chloropidae, Drosophilidae, Lauxaniidae, Muscidae, Sarcophagidae, and Tephritidae (Felska et al. 2018 and references therein).

Platytrombidium fasciatum, due to its common occurrence in Europe and relatively easy field identification of active postlarval forms, can constitute a convenient model in research on intraspecific variability of morphological traits resulting from the influence of different environmental conditions. Such inference, based on the results of laboratory rearing and affecting the earlier recognition of species boundaries, has been rarely drawn in studies of terrestrial Parasitengona mites and pertained mostly to *Dactylothrombium pulcherrimum* (Haller, 1882) (Microtrombidiidae), *Allothrombium meridionale* Berlese, 1910 (Trombidiidae), *Podothrombium filipes* C.L. Koch, 1837 (Podothrombiidae) (Mąkol 2000; Wohltmann & Gabryś 2003; Wohltmann & Mąkol 2009). In most cases, however, the scope of morphological differences between groups of larvae kept under diversified thermal conditions, has not been examined.

The aim of this study was to answer the question whether the morphology of *P. fasciatum* larvae can be altered by rearing environment, to assess the scope of morphological variation in larvae which emerge from eggs incubated in various thermal and light conditions and to determine the pool of traits that are subject to the highest variation.

Material and methods

The active postlarval forms (n=183) of *P. fasciatum* were collected in 2018 in allotments in Wrocław, Poland (51˚7'8" N; 17˚8'13" E), in fallow and near flower beds, mainly with *Vitis* sp. and (*Fragaria×ananassa*). Mites were sampled directly from the soil surface, at weekly intervals, from April to June. The material was supplemented by active postlarval forms (n=62) collected in 15 different locations in Poland, between the end of March and the beginning of July, over the period 2005–2015.

Due to the lack of well-pronounced characters that allow to distinguish between deutonymphs and adults and to select females from specimens representing active postlarval forms at macroscale (see Discussion), the total number of field-collected specimens exceeded the final representation of females in the material set for further parts of the experiment. All field-collected mites were transferred individually, with a brush, into rearing vials. The experimental rearing was carried out in glass vials (34 x ⌀ 24 mm) with semi-transparent, plastic lids, filled to 2/3 with a mixture of Plaster-of-Paris and powdered charcoal (9:1 ratio). The substrate in each vial was moistened with distilled water to maintain the humidity as indicated by the substrate color. The content of all vials was checked at regular time intervals, every two days to record the oviposition events. During control, the exposure time to conditions departing from those set for particular experiment did not exceed one minute per vial. The mites were not supplied with food during the experiment.

The specimens were preliminarily assigned to four groups (CC, CC/D, LT/D, NC/D) (for explanation of symbols and parameter settings see Table 1). Three experimental groups were kept at the laboratory, in different but constant (at 12/12h or 24h cycle) temperature and dark/light conditions, and at constant humidity parameters set to 80% RH. The fourth group, NC/D, was

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protected from light and exposed to varying thermal conditions: vials with mites were placed in the field, in the upper soil layer at a depth of about 5 cm and covered with a 3 cm layer of litter. The purpose of this part of experiment was to provide thermal and light conditions close to natural ones at the time of egg incubation. The mean air temperature in the course of experiment was 15 °C/day and 8 °C/night and the mean temperature recorded using data logger (DS1922L Maxim Integrated i-Button) placed in the upper soil layer, at a depth of about 5 cm, was 13 °C/day and 9 °C/night. Specimens assigned to the group LT/D, because of the lack of oviposition events observed two months after the introduction of the first tranche of individuals to experimental rearing, were transferred to groups NC/D and CC/D. The active postlarval forms which did not oviposit were transferred to alcohol after the termination of the experiment (July) or at specimen death.

TABLE 1. Summary of oviposition events and development time of larvae of *P. fasciatum* under different experimental conditions.

	CC	CC/D	NC/D	LT/D[1]	LT/D >> CC/D	LT/D >> NC/D
	climatic chamber (12/12 h light/dark period, 22 °C/day, 15 °C/night)	climatic chamber (dark conditions, 22 °C/day, 15 °C/night)	field (dark conditions, mean temperature in the upper soil layer 13 °C/day and 9 °C/night)	refrigerator (dark conditions, 7 °C)	(see parameters under LT/D and CC/D)	(see parameters under LT/D and NC/D)
mean diurnal temperature	18.5 °C	18.5 °C	11 °C	7 °C	7 °C >> 18.5 °C	7 °C >> 11 °C
initial sample size (total number of postlarval specimens assigned to the group)	62	64	59	60	30	30
number of females which laid eggs/other females confirmed in the experimental group	19/3	17/3	18/4	1/n/a	7/2	3/1
oviposition—larva emergence (days) (mean, range and sample size)	28 (18–44), $n_{♀}$=13	26 (19–30), $n_{♀}$=16	31 (23–45), $n_{♀}$=18	–	24 (21–24), $n_{♀}$=7	24, $n_{♀}$=2

[1] group containing specimens subsequently divided between LT/D >> CC/D and LT/D >> NC/D

Larvae were preserved in alcohol not later than two days after hatching to minimize the impact of post-hatching conditions on further inference. In morphological analyses of qualitative and quantitative traits of larvae, the slide-mounted material from groups CC, CC/D, NC/D, and LT/D >> CC/D was considered. The analyses were carried out in Nikon Eclipse E600 equipped with DIC and DS-Fi1 camera system. Measurements were taken using the NIS-Elements BR software. Damaged or obscure structures were excluded from the analyses. Morphological terminology follows Mąkol (2005), Wohltmann et al. (2007) and Felska and Mąkol (2015), with the following modifications: LB —length of idiosoma, WB—width of idiosoma, pDS min—the minimum length of posterodorsal setae on idiosoma (out of 10 randomly measured ones), pDS max—the maximum length of posterodorsal setae on idiosoma (out of 10 randomly measured ones). The explanation of all abbreviations is provided in Table 2 annotation. All measurements are given in micrometers.

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TABLE 2. Morphometric data on larvae of *P. fasciatum* obtained from eggs incubated in various laboratory conditions (for means and ranges for the combined groups see Table 5).

	CC			CC/D			NC/D			LT/D >> CC/D		
Character	n	mean	min.–max.	n	mean	min.–max.	n	mean	min.–max.	n	mean	min.–max.
LB	20	282	255–309	20	290	274–307	18	299	277–312	20	301	289–316
WB	20	168	154–187	20	182	171–195	18	178	161–192	20	183	164–202
LB/WB	20	1.68	1.58–1.79	20	1.60	1.48–1.70	18	1.69	1.57–1.92	20	1.65	1.49–1.83
LS	20	158	152–169	20	162	149–175	18	160	150–167	20	161	152–169
WS	20	120	112–127	20	120	109–130	18	120	107–125	20	119	110–125
AM	20	49	44–59	20	48	33–57	18	49	41–60	20	49	42–58
AA	20	71	62–79	20	71	56–84	18	72	61–79	20	72	62–83
AL	20	44	40–48	20	44	38–49	18	45	39–52	20	44	38–49
AW	20	110	104–117	20	111	103–120	18	112	104–120	20	110	101–117
PL	20	53	47–61	20	50	44–58	18	52	46–60	20	50	45–57
PW	20	118	112–123	20	119	111–128	18	123	114–130	20	120	111–126
S	18	77	70–83	18	76	68–85	17	74	68–81	19	74	64–82
SB	20	87	82–90	20	86	83–92	18	90	85–94	20	88	80–102
ASB	20	127	119–136	20	130	116–142	18	132	122–141	20	132	116–141
PSB	20	32	30–34	20	33	30–35	18	31	27–34	20	32	28–37
AP	20	52	48–55	20	52	44–57	18	52	48–55	20	53	49–58
MA	20	65	60–68	20	63	56–68	18	65	61–69	20	63	58–68
HS	20	43	38–47	20	42	38–48	18	42	35–49	20	44	39–50
LSS	20	141	131–151	20	138	123–151	18	141	132–148	20	143	132–157
SL	20	54	50–59	20	52	48–58	18	53	47–62	20	54	49–59
SS	19	62	55–70	20	61	54–66	18	65	57–74	20	63	54–71
pDS min	20	39	32–42	20	38	35–45	18	38	34–41	20	37	32–43
pDS max	20	54	45–60	20	53	44–58	18	53	48–60	20	53	43–62
OL	20	36	32–39	20	37	31–40	18	37	34–40	20	37	31–40
Cx I	20	57	50–61	20	57	48–63	18	58	51–67	20	57	53–62
Tr I	20	33	29–49	20	33	28–40	18	33	30–39	20	33	30–38
Fe I	20	52	48–57	20	51	48–55	18	52	49–57	20	51	47–56
Ge I	20	19	17–23	20	21	17–25	18	20	17–22	20	20	16–24
Ti I	20	44	41–49	20	44	41–50	18	43	40–48	20	43	40–47
Ta I	20	86	83–90	20	83	76–90	18	83	65–91	20	85	77–91
leg I (Cx-Ta)	20	293	283–305	20	289	275–307	18	290	268–306	20	290	282–303
Cx II	20	50	47–55	20	49	45–54	18	52	48–62	20	50	45–60
Tr II	20	28	25–31	20	29	23–37	18	28	25–32	20	30	25–35

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TABLE 2. (Continued)

	CC			CC/D			NC/D			LT/D >> CC/D		
Character	n	mean	min.–max.	n	mean	min.–max.	n	mean	min.–max.	n	mean	min.–max.
Fe II	20	47	42–51	20	46	42–48	18	48	45–52	20	47	43–52
Ge II	20	16	13–19	20	18	15–21	18	16	14–18	20	16	14–18
Ti II	20	37	33–39	20	37	32–40	18	36	34–40	20	36	32–40
Ta II	20	64	62–68	20	64	59–68	18	65	59–68	20	64	60–67
leg II (Cx-Ta)	20	242	231–251	20	242	231–256	18	245	239–257	20	243	228–253
Cx III	20	49	43–53	20	47	44–51	18	51	48–56	20	50	46–56
Tr III	20	33	30–36	20	33	28–41	18	33	30–39	20	33	31–38
Fe III	20	56	51–60	20	53	44–58	18	54	45–60	20	52	47–58
Ge III	20	17	13–20	20	20	16–23	18	17	14–20	20	17	14–20
Ti III	20	41	36–46	20	39	34–45	18	41	34–45	20	41	37–47
Ta III	20	54	50–59	20	51	44–58	18	52	47–59	20	53	48–57
leg III (Cx- Ta)	20	250	235–260	20	242	223–260	18	247	234–262	20	245	233–256
IP	20	784	757–802	20	774	745–806	18	783	750–815	20	778	751–803

AA—distance between the bases of nonsensillary setae of 1st pair (AM) on scutum; AL—length of non-sensillary seta of 2nd pair on scutum; AM—length of non-sensillary seta of 1st pair on scutum; AP—distance between the bases of AL and PL; ASB—distance between the anterior margin of scutum and the level of sensilla; AW—distance between the bases of nonsensillary setae of 2nd pair (AL) on scutum; bFe—length of basifemur; Cx—length of coxa; Ge—length of genu; HS—length of scutellum; LB—length of idiosoma; LS—length of scutum; LSS—width of scutellum; MA—distance between the bases of AM and AL; OL—length of ocular sclerite; pDS max—the maximum length of posterodorsal setae on idiosoma; pDS min—the minimum length of posterodorsal setae on idiosoma; PL—length of non-sensillary seta of 3rd pair on scutum; PSB—distance between the posterior margin of scutum and the level of posterior sensilla; PW—distance between the bases of nonsensillary setae of 3rd pair (PL) on scutum; S—length of sensillum; SB—distance between the bases of sensilla (S); SL—length of seta on scutellum; SS—distance between the bases of SL; Ta—length of tarsus; Ti—length of tibia; Tr—length of trochanter; WB—width of idiosoma; WS—width of scutum.

The statistical analyses were performed using *R* Statistical Software (R Core Team 2020). As the assumptions on multivariate normality and homogeneity of covariance matrices were not met, the MANOVA could not be applied. The impact of breeding conditions on the variability of morphological traits in larvae was tested with nonparametric, equivalent to MANOVA test. Using the ssnonpartest function in npmv 8 R-package (Burchett *et al*. 2017) we performed statistical procedure with the null hypotheses stating that distributions of random vectors are identical for all considered groups. This general hypothesis was rejected (p=0.05). To determine, which groups contribute to significant differences we used post-hoc procedure: the Kruskal-Wallis tests was applied for all dependent variables separately and the false discovery rate was controlled using the Benjamini–Hochberg stepwise adjustment. The Kruskal-Wallis test was followed by Dunn's multiple comparison for each statistically significant variable.

To transform original data from 46 dimensional space into a lower space, the Linear Discriminant Analysis (LDA), commonly used for dimensionality reduction and classification, was applied. We started analysis by removing redundant, highly correlated variables to avoid potential multicollinearity problems. As a consequence, the number of variables has been reduced from 46 to 37. The following variables were omitted from the LDA analysed: LB, WB, LS, AW, PW, leg I (Cx -Ta), leg II (Cx-Ta), leg III (Cx-Ta) and IP.

The multiple linear regression analysis was applied to explain the relationship between the development time of larvae (measured from oviposition to hatching) from experimental groups CC,

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CC/D, NC/D, LT/D >> CC/D and values of metric traits used in species diagnosing. To limit the selection of variables, only those which pertain to the dorsal shields (scutum and scutellum) were included in multivariate regression models.

Results

Altogether, 65 females, out of 245 field-collected postlarval individuals of *P. fasciatum*, laid eggs under experimental conditions. Two specimens occurred to represent the deutonymph instar, 13 confirmed females did not oviposit, for 165 individuals, identified as adults, the sex could not be ascertained due to the unavailability of clear external morphological differences between males and females (see also Discussion).

The transfer of all specimens from the group LT/D to the groups CC/D and NC/D resulted in the increase of oviposition events, expressed as seven (LT/D >> CC/D) and three (LT/D >> NC/D) cases, respectively. The summarized data on the number of oviposition events and time of larval development under different rearing conditions are provided in Table 1.

The mean development time of eggs exposed to lower temperature and lower temperature fluctuations (NC/D) was longer compared to eggs kept at laboratory regime and exposed to higher temperature and moderate temperature fluctuations (Tab. 1).

Morphometric data on larvae reared at different conditions are provided in Table 2.

TABLE 3. Morphometric traits revealing statistically significant differences (indicated with asterisk) between groups (p=0.01). Dunn's test of multiple comparisons using rank sum.

	CC/D: CC	LT/D >> CC/D: CC	NC/D: CC	LT/D >> CC/D: CC/D	NC/D: CC/D	NC/D: LT/D >> CC/D
LB		*	*	*	*	
WB	*	*	*			
LB/WB	*			*	*	
PW			*		*	*
Ge I	*			*	*	
Fe II					*	
Ge II	*			*	*	
Cx III			*	*	*	
Ta III	*		*			

Nine out of 46 analysed characters differed significantly between groups. The Kruskal-Wallis tests showed that the variables: LB, WB, LB/WB, PW, Ge I, Fe II, Ge II, Cx III, Ta III are statistically significantly different between groups where the adjusted p-values equal 0.0073, 0.1066, 0.6372, 0.0824, 0.2526, 0.2269, 0.6876, 0.0588, 0.5451, respectively. Dunn's tests showed the highest number of seven differing characters between NC/D and CC/D groups, whereas the differences between CC/D and CC, NC/D and CC, as well as LT/D >> CC/D and CC/D groups referred to five traits (Tab. 3). The characters that most frequently (four or three out of six pairwise group comparisons) showed susceptibility to different conditions between groups were LB, WB, LB/WB, PW, Ge I, Ge II, Cx III (Tab. 3).

Results of Linear Discriminant Analysis confirmed that all four group are clearly separated one from another (Fig. 1). In this three-dimensional space over 99% of total between-group variance was

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explained, thus the transformation generally preserved the variation observed in the original data set. The transformed data set, with coordinates of each point being the respective linear combinations of original traits (Tab. 4), is presented in the Figure 1. In the LDA analysis, for 26 out of 37 variables the sum of absolute values of linear discriminants (LD1, LD2 and LD3) was equal to or greater than 0.3; among the variables that had the greatest impact on the separation of groups, the LB/WB attained the highest absolute value. Six traits (LB/WB, Ge I, Fe II, Ge II, Cx III, Ta III) affecting the separation were common for the Kruskal-Wallis tests and for LDA.

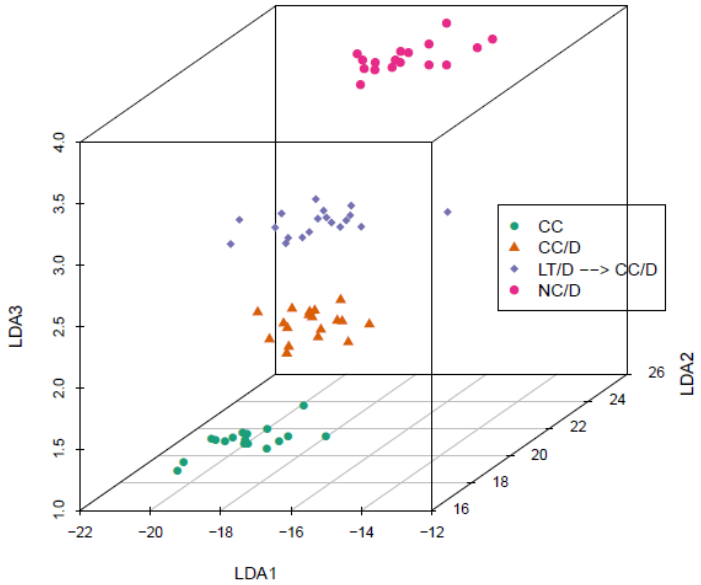


FIGURE 1. Linear Discriminant Analysis plot based on 37 input variables.

The time affected the value of three out of 16 metric traits related to scutum and scutellum (R=0.637, F=2.053, $p < 0.025$) and all of them pertained to scutum. In case of PW, the positive relationship with the duration of development was observed (b*=0.570, $p < 0.01$), whereas the negative one referred to AW (b*= -0.510, $p < 0.05$) and PSB (b*= -0.338, $p < 0.05$).

No differences in meristic traits, with special reference to the number of solenidia on various leg segments and in qualitative traits between groups, were observed.

A comparison of morphometric data on larvae from all experimental groups with the results obtained by Gabryś et al. (2005) is provided in Table 5.

Discussion

The data on the intraspecific variation of morphological traits in terrestrial Parasitengona mites have been underestimated due to the knowledge of many taxa limited to single or few specimens. The results of studies on variation in experimentally reared specimens, through an examination of a reasonably large sample size, surely widen the overall image of character range. At the same time, they give an idea of the potential impact of varied field conditions on the morphology of the species. The differences in the developmental conditions requirements between parasitengone species, reflect the variety of strategies adopted in this group of mites, being mostly related to the specific phenology, the strategy of oviposition (associated with the hibernating instar) and also, the mode of voltinism.

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TABLE 4. Coefficients of linear discriminants in LDA (for reference see the Fig. 1). Please note that the higher the absolute values, the higher the influence of the trait on the LDA.

	LD1	LD2	LD3	sum of absolute values
LB/WB	-3.96	-3.48	3.62	11.06
WS	0.06	-0.02	0.03	0.11
AM	0.07	0.11	0.01	0.19
AA	-0.02	-0.06	-0.04	0.12
AL	-0.14	0	-0.05	0.19
PL	-0.04	-0.16	0.14	0.34
S	0.01	-0.08	0	0.09
SB	0.09	0.19	0.02	0.3
ASB	0.05	0.03	0.01	0.09
PSB	0.09	-0.35	-0.09	0.53
AP	-0.37	-0.21	-0.15	0.73
MA	-0.03	-0.08	0.15	0.26
HS	-0.21	-0.09	-0.24	0.54
LSS	0.06	0.08	0.01	0.15
SL	-0.29	-0.08	-0.13	0.5
SS	-0.14	0.01	0	0.15
pDS min	0.3	-0.16	0.24	0.7
pDS max	-0.14	0.09	-0.21	0.44
OL	0.1	0.11	0.16	0.37
Cx I	0.2	-0.05	0.08	0.33
Tr I	0.14	-0.16	0.14	0.44
Fe I	0	0	0.11	0.11
Ge I	0.27	0.01	0.15	0.43
Ti I	0.03	0.09	0.11	0.23
Ta I	-0.22	-0.18	-0.19	0.59
Cx II	-0.22	0.11	-0.04	0.37
Tr II	0.08	0.17	-0.06	0.31
Fe II	-0.09	-0.09	0.25	0.43
Ge II	0.48	0.27	-0.05	0.8
Ti II	-0.12	-0.2	0.04	0.36
Ta II	0.17	0.35	-0.13	0.65
Cx III	0.03	0.28	0.08	0.39
Tr III	0.04	0.11	0.15	0.3
Fe III	0.16	-0.17	0.19	0.52
Ge III	0.26	-0.15	0.14	0.55
Ti III	-0.25	-0.07	-0.15	0.47
Ta III	-0.13	-0.11	-0.06	0.3

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TABLE 5. Morphometric data on larvae of *P. fasciatum*.

	Gabryś et al. (2005)[1]				present study			
character	n	mean	min.–max.	s	n	mean	min.–max.	s
LB	12	291	280–309	7.81	78	293	255–316	14.03
WB	13	176	160–196	10.85	78	177	154–202	11.09
LB/WB	12	1.67	1.5–1.8	0.09	78	1.65	1.5–1.9	0.09
LS	10	156	150–162	4.17	78	160	149–175	5.24
WS	10	119	108–125	5.15	78	120	107–130	4.73
AM	18	52	44–63	6.58	78	49	33–60	5.17
AA	21	68	62–78	4.68	78	72	56–84	5.39
AL	22	45	40–55	3.95	78	44	38–52	3.34
AW	21	107	98–119	6.37	78	111	101–120	4.42
PL	23	52	46–59	3.04	78	52	44–61	3.69
PW	23	117	100–131	7.78	78	120	111–130	4.07
S	24	76	70–82	3.51	72	75	64–85	4.29
SB	21	84	75–93	5.39	78	88	80–102	3.36
ASB	25	127	115–140	6.01	78	130	116–142	6.39
PSB	25	32	30–40	2.40	78	32	27–37	1.76
AP	20	49	45–53	2.30	78	52	44–58	2.13
MA	23	62	58–66	1.90	78	64	56–69	2.94
HS	15	42	39–45	1.81	78	43	35–50	2.87
LSS	14	145	133–160	6.72	78	141	123–157	5.98
SL	15	54	50–58	2.30	78	53	47–62	3.04
SS	25	59	53–64	3.84	77	63	54–74	4.32
Cx I	25	56	46–64	7.02	78	58	48–67	2.87
Tr I	25	34	29–39	3.26	78	33	28–39	2.49
Fe I	25	51	45–56	2.93	78	52	47–57	2.17
Ge I	25	20	18–22	1.19	78	20	16–25	1.89
Ti I	25	44	41–49	1.95	78	44	40–50	2.19
Ta I	25	82	75–90	3.55	78	84	65–91	4.19
leg I (Cx-Ta)	25	287	260–310	15.47	78	291	268–307	7.36
Cx II	25	53	44–61	6.54	78	50	45–62	2.96
Tr II	25	29	25–35	2.68	78	29	23–37	2.73
Fe II	25	47	43–51	2.59	78	47	42–52	2.39
Ge II	25	17	14–19	1.32	78	17	13–21	1.65
Ti II	25	36	33–39	1.59	78	36	32–40	1.95
Ta II	25	62	59–67	2.15	78	64	59–68	2.23
leg II (Cx-Ta)	25	244	224–261	12.38	78	243	228–257	5.78
Cx III	25	50	44–56	4.35	78	49	43–56	2.96
Tr III	25	35	31–39	2.13	78	33	28–41	2.12
Fe III	25	53	47–60	2.70	78	54	44–60	3.45
Ge III	25	18	13–20	1.68	78	18	13–23	2.37
Ti III	25	41	37–45	2.61	78	40	34–47	2.87
Ta III	25	52	48–58	2.75	78	52	44–59	3.17
leg III (Cx-Ta)	25	248	234–260	8.25	78	246	223–262	7.43
IP	25	805	758–852	26.95	78	780	745–815	15.29

[1] rounded to integers (except for ratios)

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The scope of variation observed in experimentally reared specimens, but also the probability of the occurrence of anomalous states is likely to be more pronounced in instars that develop from hibernating or diapausing stages (Mąkol 2000; Wohltmann & Mąkol 2009). On the other hand, any conditions deviating from the natural requirements of the species may result in developmental disorders, which are also reflected in the morphology. The frequency of this phenomenon in natural conditions remains unknown. Wohltmann and Mąkol (2009) observed the morphological abnormalities of palps and/or setal pattern on scutum in larvae of *Allothrombium meridionale* (Trombidiidae), which hatched under laboratory conditions from eggs not exposed to lower temperatures. Such modifications were not observed in larvae that hatched from eggs after chilling, which is consistent with the knowledge of *A. meridionale* as species, in which eggs undergo an obligatory diapause.

In *P. fasciatum* the deutonymphs and adults are subject to diapause (Wohltmann 2000); it may explain the relatively low variation observed in metric characters of larvae, in the absence of qualitative traits variability exceeding the hitherto knowledge of the species but also in the absence of any observed anomalies.

In our experiment, the time of collecting the specimens in the field, as well as the period between the transfer of females to laboratory and the egg laying could have an impact on the overall female fitness, which in turn could affect the development and morphology of larval instar. To limit the impact of these variables, the similar studies, in which the different conditions are set starting from oviposition event, should be carried out in the future.

Nonetheless, the experimental rearing of microtrombidiid mites, for which a certain representation of females constitutes a founding group, should involve the collection of three- to four times as many postlarval individuals in the field. The differences between the deutonymphs and adults have long posed the difficulties in unambiguous identification of instar in the field, whereas the secondary sexual differences in adults of Microtrombidiidae cannot be ascertained based on the structure and chaetotaxy of genital valves. The latter had its effect on the representation of females in the group of field-collected specimens. Feider (1959), who examined the sexual characters in Trombidioidea s. lato, referred to these limitations in contrast to the traits observed in several other families of terrestrial Parasitengona mites. Some differences pointed to by Feider (1959) may pertain to the structure of the uropore, which in many cases was protruded above the idiosoma surface. Due to the variability observed, we could not confirm this difference as one that allows the certain identification of the sex. The same problem might have been approached by those authors, who referred to adult specimens in studies of microtrombidiid mites, without further inference on sex. The latter, however, stays beyond the scope of the present manuscript and should be subject to separate analyses.

The influence of temperature on the values of metric traits was most strongly expressed in relation to six (out of 46) traits, which turned out to be common for both statistical methods applied (MANOVA, LDA). The cumulative development time required by eggs to transform into prelarvae and then—for larval emergence was on average 27 days and varied between 18 and 45 days. It attained the highest value in samples that were subject to the relatively low mean daily temperature (11 °C) and temperature fluctuations in day/night cycle (4 °C), over the entire period of egg incubation (NC/D group). In studies carried out by Gabryś et al. (2005) the eggs developed into larvae in 23 days (19–25, n=3 clutches) at 22–25 °C, in 28 days (22–30, n=10 clutches) at 20 °C and in 54 days (52–55, n=3 clutches) at 15 °C. Wohltmann (2000) reported the development time of eggs into larvae as varying between 27 and 30 days at 20 °C.

The larger diurnal temperature fluctuations (7 °C) at higher mean daily temperature (18.5 °C) resulted in still shorter period of egg incubation compared to treatment with lower temperature fluctuations in day/night cycle (4 °C) and the lower mean daily temperature (11 °C). The latter

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suggests that the moderate temperature fluctuations during egg incubation, at least as far as non-diapausing eggs of *P. fasciatum* are concerned, do not affect the overall time required for the transition to larva, whereas the mean diurnal temperature at moderate temperature fluctuations seems to play a crucial role. The latter, in the deficiency of similar studies related to terrestrial parasitengone mites, is congruent with the results obtained by Xing *et al*. (2015) who studied the effects of temperature fluctuations at the same mean temperature on the development of the diamondback moth (Lepidoptera: Plutellidae).

The longer duration of larval development, which was related to the mean temperature over the entire incubation period, had only a meagre effect on the values of metric characters related to scutum and scutellum. A gradual widening of the scutum at the level of the third pair of non-sensillary setae was associated with the decrease in distance between the bases of the second pair of non-sensillary setae and with the shortening of the sclerite length, behind the level of sensilla.

Dos Santos Costa *et al.* (2019) pointed to the smaller size of experimentally reared specimens (deutonymphs) of *Charletonia rocciai* Treat & Flechtmann, 1979 (Erythraeidae), compared to field-collected one, expressed in the shorter legs, crista, and sensilla. The limited sample size, however, did not allow the same authors the further conclusions on the actual reason for these differences. It cannot be excluded that such a phenomenon may be caused by the earlier detachment of larvae from the host as a result of stimuli that were triggered during field collection, it may as well result from parasitism of the larva under suboptimal conditions for the host in the laboratory

In no case, the effect of light/dark period on the development could have been confirmed, which corroborates also the observations of Eggers (1995) on *Johnstoniana errans* (Johnston, 1852) (Johnstonianidae) and Zawal *et al.* (2018) on the water mite *Eylais extendens* (Eylaidae).

Morphometric data on larvae reared at different conditions broaden the knowledge of the intraspecific variation of measurable traits in *P. fasciatum*. In case of 33 characters (out of 43—see Table 5), the wider range of variation was observed and for seven other characters the variability going beyond the hitherto knowledge of the species was noted, compared to data provided by Gabryś *et al*. (2005). The latter, however, may result also from the sample size (10–25, depending on the character, in the results provided by Gabryś *et al.* 2005; 72–78 in our study). The intraspecific variation, facilitated both by the increased sample size and the more diverse environmental conditions, may reflect the potential of the species for adaptation in new environments.

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