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## Morphological variation of *Varroa destructor* (Parasitiformes, Varroidae) in different seasons

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Varroosis is one of the most dangerous and common diseases of honey bees (*Apis mellifera* Linnaeus, 1758) worldwide, caused by gamasid mites of the species *Varroa destructor* Anderson and Trueman, 2000. This external parasite is widespread and adapted to the climate conditions of most countries of the world, and it can infect bees at any life stage. It parasitizes on worker bees, male and queen bees, larvae and pupae, feeding on their hemolymph and fat bodies, causing lower survival rates and lower density of bee colonies, decreasing the bees' life span. Here, we studied the specifics of the seasonal variation of female *V. destructor* mites, obtained from honey bees, by the morphological characters of mites belonging to the summer and winter generations, and their differences were established. Using the methods of multivariate statistics, we found significant differences between the summer (June–July) and winter (October–November) morphotypes of *V. destructor* mites. There are differences between the seasonal samples by 12 morphological characters of the parasite, namely the width of dorsal shield, width of dorsoventral shield, number of pores on sternal shield, length of tarsus and macrochaeta IV, and distances between setae of gnathosoma. Processing the seasonal samples of mites with discriminant analysis resulted in differences by 11 morphological characters including the length of dorsal shield, number of lancet setae, length and width of genitoventral shield, width of anal shield, number of setae and pores on sternal shield and distance between setae of gnathosoma. In general, the summer females are smaller and elongated compared to winter females, with larger genitoventral shield and shorter legs. The mites of summer and winter generations are adapted to different seasons: the summer mites to the reproductive period, the winter generation to overwintering on bees. The ratio of morphotypes in female *V. destructor* mites is observed to change during the year, from 20.2% winter morphotype in summer generation to 20.7% summer morphotype in winter mites. Studying the influence of acaricides on the distinguished morphotypes is a promising approach to improve pest control measures against varroosis of honey bees.

**Keywords:** varroosis; *Apis mellifera*; female mites; morphotype; seasonal adaptation.

### Introduction

The honey bees evolved as eusocial animals to better adapt to their habitat conditions and widen their species range significantly (Kovalskiy et al., 2018; Kovalchuk et al., 2019; Vishchur et al., 2019). One of the adaptations was the genetically determined necessity to live as a whole complex super-organism, a bee colony. Not a single one of its structural elements (the queen, workers and male bees) can survive on its own. This life form ensured the continued success of these bees as a species. The colony is composed of thousands of specimens per one queen, and it can persist in the most unfavourable environmental conditions and still succeed in reproducing (Delaney et al., 2010; Bloch & Grozinger, 2011; Hewlett et al., 2018; Shell & Rehan, 2018). The honey industry struggles with huge economic losses caused by the infectious and non-infectious diseases of bees and their offspring. Varroosis is one of such afflictions which pose a global problem for apiculture, weakening and reducing the number of bee colonies, negatively affecting the environment and decreasing the yield of entomophilous crops and overall productivity of the industry (Boecking & Genersch, 2008; Dahle, 2010; Rosenkranz et al., 2010; Nazzi et al., 2012; Affognon et al., 2015). In long-term studies it was confirmed that varroosis is the most widely spread mite infection of honey bees of the species *Apis mellifera* Linnaeus, 1758. The infection can become panzootic, acting in all habitats the mites can possibly colonize (Akimov et al., 2004; Le Conte et al., 2010; Akinwande et al., 2012; Nazzi & Le Conte, 2016; Rinkevich et al., 2017).

The bee parasites of the genus *Varroa* Oudemans, 1904 are members of the family Varroidae which infect colonies of bees of the genus *Apis* Linnaeus, 1758. There are six species of two genera of these specialized mites: *V. destructor* Anderson et Trueman, 2000, *V. jacobsoni* Oudemans, 1904, *V. underwoodi* Delfinado-Baker et Aggarwal, 1987, *V. rindereri* De Gurman et Delfinado-Baker, 1996, *Euvarroa sinhai* Delfinado et Baker, 1974, *E. wongsirii* Lekprayoon et Tangkanasing, 1991 (Oudemans, 1904; Woo, 1992; Anderson et al., 1997; Anderson & Trueman, 2000; Dadgostar & Nozari, 2018). *Varroa destructor*, *V. jacobsoni* and *V. underwoodi* can also infect colonies of *Apis c. cerana* Fabricius, 1793 bees. *V. rindereri* only parasitizes *A. koschevnikovi* Enderlein, 1906 bees on Borneo and Philippine islands (Anderson & Trueman, 2000). *Euvarroa sinhai* infects bees of the species *A. florea* Fabricius, 1787 and *A. mellifera*, and it is recorded in India, Sri Lanka, Thailand and Iran (Mossadegh, 1990; Akimov et al., 1993). The mite species *E. wongsirii* Lekprayoon et Tangkanasing, 1991 was found on *A. andreniformis* Smith, 1858 bees in Thailand, Malaysia, India and Indonesia (Lekprayoon & Tangkanasing, 1991; Otis & Kraly, 2001).

However, it has been confirmed, that the most frequent parasites of *A. mellifera* honey bees are mites of the species *V. destructor*. Their life cycle includes the obligate change of hosts of different stages of development and physiological state. Hence, the mites do not parasitize single specimens of bees, but the whole colony. This is confirmed also by the fact that the development of varroosis results not in death of single bees, but all bees of a colony. This kind of parasitism is induced

by the relative stability of the beehive conditions, sustained by bees even in winter. That is the reason for the high pathogenicity of *V. destructor* mites to the honey bees, and for the complicated pest control measures (Akimov et al., 1988; Akimov & Kiryushyn, 2010; Akimov & Korzh, 2012; Oddie et al., 2019).

It is known that *Varroa destructor* (= *Varroa jacobsoni* auct.) was found for the first time in nests of the Indian honey bee, *Apis cerana indica* Fabricius, 1798. Both species (*V. destructor* and *A. cerana*) exist in ecological balance with each other (Peng et al., 1987; Rath, 1999; Oldroyd, 1999; Nanork et al., 2007). However, the anthropogenic influence on the natural habitats of *A. cerana* and the introduction of the more productive honey bee species, *A. mellifera*, allowed the parasitic species to infect the latter bee, significantly increasing the mite's range (Delfinado, 1963; Griffiths & Bowman, 1981; Kuznetsov, 2005; Kuznetsov & Lelej, 2005).

The expansion of this dangerous parasite, which successfully adapted to a new host and new climate zones, is why the intraspecific morphological differentiation of *V. destructor* is of scientific interest. Thus, there have been reports of morphological variability of mites from different host species and continents (Delfinado-Baker, 1988; Delfinado-Baker & Houk, 1989; Akimov & Benedyk, 2004). Also, there are observations of intraspecific regional complexes of *V. destructor* and the sexual and seasonal morphological variations of mites. The seasonal variation is described as numbers of anomalies in the studied characters of *V. destructor* females, which increase during the spring and summer, and decrease from summer to autumn (Delfinado & Houck, 1989; Akimov et al., 2004; Maggi et al., 2009; Abou-Shaara & Tabikha, 2017). Although two mite morphotypes (summer and winter) have been distinguished in previous studies, no strong differentiation has been described for them. Hence, the morphological characters of *V. destructor* mites which parasitize on *A. mellifera* bees in different seasons should be studied in detail. That will allow us to determine the mite's capability of adaptation, and to develop new morphotype-specific approaches in order to improve the effectiveness of pest control measures. The aim of the present work was to study the seasonal specifics of variation in *V. destructor* by the morphological characters of mites from the summer and winter generations, and to find out previously undiscovered differences between them.

## Materials and methods

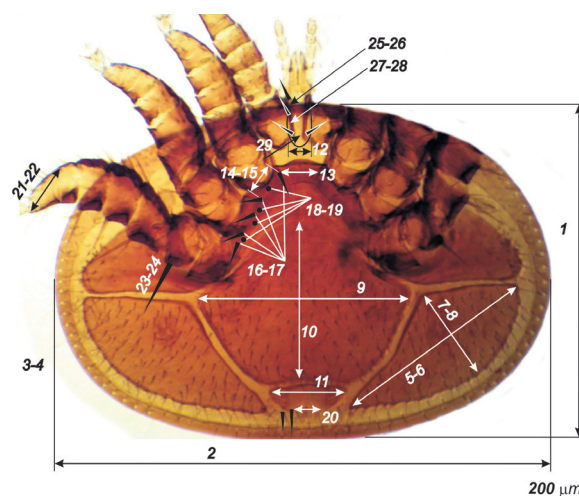
The specifics of morphology and variation of *V. destructor* females were studied on samples of mites collected in 2018 in colonies of *A. mellifera* bees during two seasons, summer (June and July) and winter (October and November). Mites were collected at private honey farms in Poltava region of Ukraine. Mite specimens were fixed in 70% ethanol and mounted on permanent slides in Hoyer's mounting medium (Zahvatkin, 1941). Biometrical studies of *V. destructor* females were conducted using a Biolam L-211 microscope. Each sample consisted of 120 female mites, 240 mites were measured in total. The morphological variation was studied by the metrical and qualitative characters, 10 of which paired. Measurements were taken from both the level and right sides of body. Overall, data on 19 morphological characters was statistically processed (Fig. 1).

Statistical processing of data was conducted using the method of principal components, one-way dispersion analysis and the Tukey test. The calculations were done using the statistical packets PAST 3 (Hammer et al., 2001) and Statistica 12 for Windows (StatSoft, Inc., USA).

## Results

According to the eigenvalues (PCA), the first three components explain 68.6% of the total variance (Table 1). Analysis of eigenvalues of the first (I) principal component, which describes 50.1% of the total variance, shows that the main positive values belong to the size of the dorsal shield (characters 1 and 2), sizes of shields on the ventral side of body (characters 5–6, 9–10). The second (II) principal component explains 11.2% of the total variance, and the major positive values in it are of the length of the dorsal shield (character 1), sizes of shields on the ventral side of the body (characters 5–6, 8, 11), width of the gnathosome (character 12), and distance between the third pair of setae of the

gnathosome (character 29). The maximum negative value in that component belongs to the character 2, the width of the dorsal shield. The third (III) principal component explains only 7.3% of the total variance. Its main values belong to the size of the genitoventral shield (character 9–10), and distance between the third pair of setae of the gnathosome (character 29).



**Fig. 1.** Morphological characters of female *Varroa destructor* mites: 1 – length of dorsal shield; 2 – width of dorsal shield; 3–4 – number of lancet setae; 5–6 – width of pleural shield; 7–8 – length of pleural shield; 9 – width of genitoventral shield; 10 – length of genitoventral shield; 11 – width of anal shield; 12 – width of gnathosome base; 13 – distance between the first pair of setae of sternal shield; 14–15 – distance between the first and second pairs of setae of sternal shield; 16–17 – number of setae on sternal shield; 18–19 – number of pores on sternal shield; 20 – distance between anal setae; 21–22 – length of tarsus IV; 23–24 – length of macrochaeta IV; 25–26 – distance between first and second setae of hypostome; 27–28 – distance between second and third setae of hypostome; 29 – distance between third pairs of setae of hypostome

On the scatterplots in the space of three principal components it can be seen that the data from the different seasonal samples overlap strongly. That is, they are not distinguished in the space of these components (Fig. 2).

The next stage of analysis was changing the absolute values of characters with their average values, excluding all quantitative characters (2–3, 16–19). Only one principal component was obtained then, explaining 100% of the total considered variance. The main positive values in the difference between the summer and winter mite generations belonged to the following characters: length of dorsal shield (character 1), length and width of genitoventral shield (characters 9, 10), distance between second and third setae of hypostome (characters 27–28). The component's value was negatively affected by the width of dorsal shield (character 2) and length of macrochaeta IV (characters 23, 24) (Table 2).

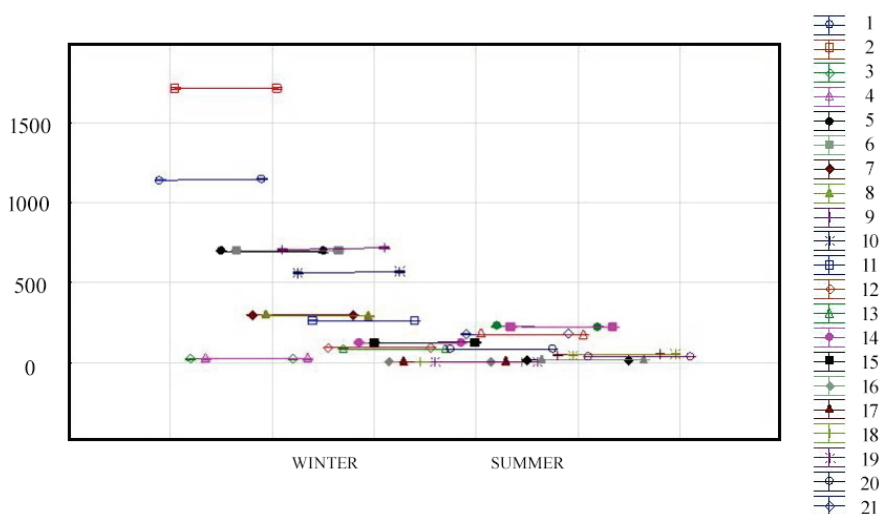
Interestingly, the increasing length of the dorsal shield is associated with the decreasing width of the dorsal shield, according to that component. The winter and summer generations of mites do not overlap in the space of the first principal component. Considering the eigenvalues in that component, it can be said that the summer females are smaller, more elongated, with larger genitoventral shield and shorter legs. The winter mites are, in contrast, flatter, with smaller genitoventral shield and longer legs.

The average values of morphological characters of mites sampled in winter and summer were compared using the Tukey test. The results showed differences between these samples by 12 morphological characters (Table 3). These are, firstly, width of dorsal shield (character 1), width of dorsoventral shield (character 9), number of pores on the sternal shield (characters 18, 19), length of tarsus and macrochaeta IV (characters 21–24) and distances between the setae of gnathosome (characters 25, 27–29). Analysis of the obtained data showed that the winter females are elongated, with smaller genitoventral shield, more pores on the sternal shield and longer legs. Summer samples are, by contrast, wider with wider dorsal shield and shorter legs.

**Table 1**Eigenvalues of morphological characters of *Varroa destructor* female mites of seasonal samples in I, II and III principal components

Character	Principal component			Character	Principal component		
	I	II	III		I	II	III
1	0.2685**	0.2694**	-0.0890	15	0.0068	-0.0181	-0.0007
2	0.7140***	-0.6671***	0.1371	20	0.0016	-0.0062	0.0073
5	0.3859*	0.3852*	0.1052	21	0.0282	0.0018	-0.0051
6	0.3640***	0.3978*	0.1153	22	0.0240	0.0006	-0.0446
7	0.1213	0.1343	0.0758	23	0.0177	-0.0371	-0.0255
8	0.1012	0.4276**	-0.1271	24	0.0260	-0.0032	0.0024
9	0.2603**	0.0270	0.3676***	25	0.0019	-0.0054	-0.0017
10	0.1980**	0.1380	0.8587***	26	0.0041	-0.0094	0.0199
11	0.0297	0.2542*	-0.1599	27	0.0045	-0.0037	0.0050
12	0.0136	0.2563**	-0.1065	28	-0.0001	0.0062	0.0017
13	0.0080	-0.0383	0.0450	29	-0.0002	0.3420**	0.3271**
14	0.0351	-0.0004	0.0559	% of total variance	50.072	11.1910	7.3070

Note: significance levels \* –  $P < 0.05$ , \*\* –  $P < 0.01$ , \*\*\* –  $P < 0.001$ .

**Fig. 2.** Scatterplot of average values of morphological characters in summer and winter female *Varroa destructor* mites**Table 2**Eigenvalues of morphological characters of female *Varroa destructor* mites of seasonal samples in I principal component

Character	Principal component I	Character	Principal component I
1	0.263***	15	0.131
2	-0.603**	20	-0.004
5	-0.036	21	-0.076
6	-0.108	22	0.080
7	-0.020	23	-0.135**
8	-0.129	24	-0.164***
9	0.550***	25	0.031
10	0.207**	26	-0.009
11	-0.091	27	0.170***
12	-0.067	28	0.156**
13	-0.031	29	0.040
14	0.038	% of total variance	1.000

Note: significance levels \*\* –  $P < 0.01$ , \*\*\* –  $P < 0.001$ .

The morphological characters of mites of the seasonal samples were analyzed with discriminant analysis. The results showed that the difference between samples is statistically significant (Wilks' Lambda = 0.539;  $P = 1.2 \cdot 10^{-5}$ ). Only one canonical axis was determined with the total dispersion of 0.972%.

Thus, the seasonal samples are statistically different by 11 morphological characters. The analysis of coefficients with the first and only canonical axis showed that the winter and summer mites are different by the following characters: length of dorsal shield (character 1), number of lancet setae (character 4), length and width of genitoventral shield (characters 9, 10), width of anal shield (character 11), number of setae and pores of sternal shield (characters 16, 18, 19) and distance between the gnathosome setae (characters 27–29) (Table 4).

**Table 3**Average values of morphological characters and results of comparison of female *Varroa destructor* mites in seasonal samples

Character	Winter	Summer	Winter-summer
1	1140	1146	*
2	1714	1712	–
7	298	297	–
8	295	292	–
9	705	719	***
10	561	566	–
11	263	260	–
18	6.4	5.9	***
19	6.3	5.9	*
20	6.5	6.0	–
21	179	177	***
22	178	176	***
23	226	223	*
24	225	221	*
25	19	20	*
26	19	19	–
27	49	53	***
28	49	53	***
29	39	40	**

Note: significance levels \* –  $P < 0.05$ , \*\* –  $P < 0.01$ , \*\*\* –  $P < 0.001$ , – significance levels  $P > 0.05$ .

Along the axis, the body of mite becomes more elongated and the number of pores on the ventral shield decreases. Thus, it can be said that characteristics of the summer morphotype are found farther from the starting point of the axis.

Analysis of the classification matrix showed that there are 20.7% of mites of summer morphotype in the winter generation, and 20.2% of mites with winter morphotype in the summer generation (Table 5).



**Table 4**  
Results of discriminant analysis of morphological characters of female *Varroa destructor* in seasonal samples

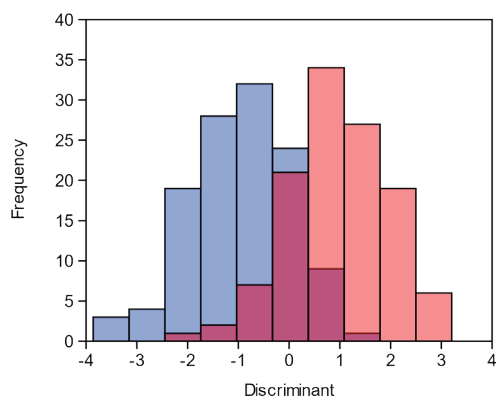
Characters	Winter – summer	Characters	Winter – summer
1	3.157**	16	0.065*
2	-0.743	17	-0.027
3	-0.019	18	-0.289***
4	0.080**	19	-0.181***
5	-0.832	20	-0.049
6	-2.038	21	-0.972
7	-0.194	22	-1.056
8	-1.839	23	-1.742
9	6.537***	24	-2.088
10	2.288**	25	0.363
11	-1.146***	26	-0.120
12	-0.780	27	2.101***
13	-0.326	28	1.913***
14	0.570	29	0.543*
15	1.445	Total, %	1.000

Note: significance levels \* –  $P < 0.05$ , \*\* –  $P < 0.01$ , \*\*\* –  $P < 0.001$ .

**Table 5**  
Classification matrix of the female *Varroa destructor* of seasonal samples

Groups	Winter	Summer	Total
Winter	96	25	121
Summer	24	95	119
Total	120	120	240

The plot of mites of summer and winter generations is shown on Figure 3. It can be seen that the studied samples are clearly distinguished, yet in each morphotype there are mites of the other generation.



**Fig. 3.** Distribution of *Varroa destructor* females of winter and summer generations (winter sample is to the left, summer sample is to the right)

The results of dispersion analysis of the morphological characters of seasonal mite samples showed that the dispersion between the winter and summer females is higher in summer mites by eight characters (number of pores of sternal shield (18–19); length of tarsus IV (21–22); length of macrochaeta IV (23–24); distance between second and third setae of hypostome (27); distance between the third pairs of setae of hypostome (29)). In contrast, the dispersion is higher in winter mites only by four characters (length of dorsal shield (1); width of genitoventral shield (9); distance between the first and second setae of hypostome (25); distance between the second and third setae of hypostome (28), Hotelling's 204.1;  $P < 0.001$ ). Thus the dispersion of characters is higher in summer mites by more characters compared to the winter generation of mites (Table 6).

Hence, the data, obtained with various methods of variance statistics, show the significant differences between the summer and winter morphotypes of *V. destructor* female mites by a complex of morphological characters. Moreover, the ratio of these morphotypes changes regularly in different seasons.

## Discussion

According to our study, there are significant differences between *V. destructor* females of winter and summer generations by a complex of morphological characters. The summer generation of mites is charac-

terized by smaller sizes, transversally elongated body, larger genitoventral shield, fewer pores of the sternal shield, shorter legs and high dispersion of most of the characters. The winter mites, conversely, are larger, elongated, with smaller genitoventral shield, more pores of the sternal shield, longer legs, and their characters are more stable. The highest number of differences between the mites of winter and summer samples is found using the dispersion analysis: there are statistically significant differences by 12 morphological characters: width of dorsal shield (character 1), width of dorsoventral shield (character 9), number of pores of sternal shield (characters 18, 19), length of tarsus and macrochaeta IV (characters 21–24), distance between the gnathosome setae (characters 25, 27–29).

**Table 6**  
Comparison of characters of *Varroa destructor* females in seasonal samples

Characters	Winter	Summer	Characters	Winter	Summer
1	24.24***	22.36	23	11.09	13.35***
9	27.12**	19.14	24	12.62	13.08**
18	1.10	1.26**	25	2.57**	2.09
19	1.02	1.49*	27	6.25	6.48**
21	3.40	4.66**	28	6.39**	6.19
22	3.80	5.13***	29	2.82	3.20***

Note: significance levels \* –  $P < 0.05$ , \*\* –  $P < 0.01$ , \*\*\* –  $P < 0.001$ .

According to the literature data, even though the external parasite *V. destructor* is almost cosmopolite, it is affected by various environmental conditions mostly indirectly, through the microclimate conditions of beehives. The full life cycle of the parasitic specimens occurs in the sealed honeycombs (Adjlane et al., 2015; Seeley, 2017). Hence, the summer mites obviously have some characters by which they are better adapted to inhabiting the beehive cells in summer. The winter mites have adaptations with which they successfully reproduce in winter. There are reports of two morphotypes, summer and winter, in females of *V. destructor* mites. It has been confirmed that these mite generations differ by a complex of morphological characters, and have differing survivability in different seasons, including unequal stability of development. It has also been found that the mites of summer generation are characterized by higher dispersion of morphological characters, while characters of the winter mites are more stable (Akimov et al., 1989, 1991, 1993).

It is known that, despite the relatively stable environmental conditions of bees of the genus *Apis*, the reproduction activity of bees is different in winter and summer (Singh, 1962). Hence, mites stay on bees and are affected by environmental factors. Conversely, the mites which adapted to parasitism in bee cells (used for reproduction) are benefitting in more favourable conditions and reproduce better. That could have induced the specialization of the parasite and result in the development of two morphotypes. It has been shown that in *V. destructor* these morphotypes occur simultaneously (Akimov et al., 1988; Akimov & Zaloznaya, 1996; Anderson, 2000; Maggi et al., 2009). We confirm that in our study, having found 20.7% of summer mites in the winter generation of mites, and 20.2% of winter mites in the summer generation of mites. The ratio of seasonal morphotypes in bees can be determined by different timing of their reproduction, affected by the selected variety of honey bees and the local climate conditions.

It is known that the eigenvalues of same mode in a component point to the one factor influencing the size variation of mites. That factor causes the increasing or decreasing of values for the whole complex of characters. In our work, these eigenvalues were mostly of different directions, which can point to an independent reason that affects the characters unequally. Hence, the reasons driving the changes of different morphological characters can be also different, and determined by the species' adaptation to new conditions. That adaptation can result in various forms of the morphological variation (sexual, seasonal and geographic) in that mite species. Previously it has been found that the geographic and seasonal variations manifest commensurably, meaning that the range of geographic variation of *V. destructor* characters can be found in one colony (Delfinado-Baker & Houk, 1989; Aude et al., 2016; Dadgostar & Nozari, 2018). Recently it was also established that the variation of mites' morphology was realized through the variation of sizes of legs, shields of ventral side, and mite body.

That variation is the base of the morphological changes of *V. destructor* mites during their expansion to new areas (Giménez Martínez et al., 2017; Farjamfar et al., 2018). Thus, we can assume that the seasonal variation is that mobilization reserve which allowed the parasite to persist through the cyclic changes of seasons due to its colonizing a new host species and expand its range significantly.

All that can notably affect the development of new pest control measures for different seasons. Another matter to consider is the different susceptibility of summer and overwintered mites to the acaricide preparations. In different seasons, mites have different sensitivity to the chemical preparations and are unequally affected by them (Maggi et al., 2012; van Dooremalen et al., 2012). For example, the ratio of winter and summer forms in the population can be driven by either of them, in our opinion. This is even more important because the ratio of morphotypes can be changed by enhanced treatment with preparations at the moment of prevalence of a certain form. Then, the balance of forms can be disturbed. Thus, a more discerning approach to pest control should be implemented to prevent and control cases of varroosis.

## Conclusion

The study of morphological variation of female *V. destructor* mites obtained from *A. mellifera* honey bees showed the significant differences between the specimens of different seasonal populations, which confirms the presence of two morphotypes of this parasitic species, the winter and the summer ones. We established the complexes of morphological characters distinguishing the summer and winter female mites. Summer females are smaller in size, elongated transversally, with larger genitoventral shield and shorter legs. Winter mites are flatter, with smaller genitoventral shield and longer legs. The observed morphotypes of *V. destructor* point to their ability for seasonal variation, with which the winter mites are better adapted to overwintering on bees, and summer mites are more suited for reproduction in bee cells during the bees' reproductive season. The morphotypes exist simultaneously in a balance described by a stable ratio. That is confirmed by our observations of 20.7% of summer mites in a winter population and 20.2% winter mites in the summer sample. These data on the presence of different morphotypes in the parasitic species require a more discerning approach to the prophylactic measures in different seasons.

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