# MORPHOLOGICAL AND ECOLOGICAL CHARACTERISTICS OF THE HYBRID LARVAE AND NYMPHS OF THE SHEEP (*IXODES RICINUS* L., 1758) AND TAIGA (*IXODES PERSULCATUS* SCH., 1930) TICKS (ACARI: IXODIDAE)

### Ludmila A. Grigoryeva<sup>1</sup>, Andrey B. Shatrov<sup>1</sup> and Anastasya V. Zaytseva<sup>2</sup>

<sup>1</sup>Zoological Institute of the Russian Academy of Sciences, St. Petersburg, Russia <sup>2</sup>St. Petersburg State University of Veterinary Medicine, St. Petersburg, Russia \*Corresponding author; e-mail: ludmila.grigoryeva@zin.ru

ABSTRACT: The external morphology of the larvae and nymphs of the interspecific hybrids of the European forest and taiga ticks (*Ixodes ricinus* (L., 1758) and *I. persulcatus* Sch., 1930, respectively), taken from laboratory culture, was examined with scanning electron microscopy (SEM). Our article presents a comparative diagnosis of the hybrids and lists their differences from the parent species. Our analysis is based on the size and the number of marginal, premarginal and median setae on the dorsal body side; epimeral and adanal setae on the ventral body side in nymphs; and marginal setae in larvae. The ecological characteristics of the larvae and nymphs of the interspecific hybrids in the natural inserts were also observed. The results show that the lifespan of larvae F1  $\bigcirc$  *I. persulcatus* ×  $\bigcirc$  *I. ricinus* is 14–16 months, F1  $\bigcirc$  *I. persulcatus*—12–15 months; for the nymphs, the life expectancy in F1  $\bigcirc$  *I. persulcatus* ×  $\bigcirc$  *I. ricinus* is 14–16 months, F1  $\bigcirc$  *I. ricinus* ×  $\bigcirc$  *I. persulcatus*—12–15 months.

KEY WORDS: Ixodes ricinus, Ixodes persulcatus, hybrids, larvae, nymphs, morphology, ecology

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#### INTRODUCTION

Ixodid ticks *Ixodes ricinus* (L., 1758)) and *I. persulcatus* Sch., 1930 are reservoirs and vectors of many dangerous pathogens of medical and veterinary importance. They are an important component of the natural foci of vector-borne infections, such as the transmission-borne encephalitis (TBE), Lyme disease (TBB), granulocytic anaplasmosis, monocytotrophic ehrlichiosis, tularemia, Q-rickettsiosis and some others (Korenberg *et al.* 2013; Tokarevich *et al.* 2019; Grigoryeva *et al.* 2019).

The habitation range of I. persulcatus is a continuous area that encompasses the southern part of the central taiga, as well as the entire southern taiga subzone of Eurasia. The range of I. ricinus covers all countries of Western, Central and Southern Europe and extends into northern Africa and the Middle East. The eastern border of this species' range is in Russia, where it occupies a large area extending from the western regions of the country until Middle Volga (Filippova 1977, 1990, 2002; Kolonin 1981; Hillyard 1996; Gugliemone et al. 2014). The ranges of the two aforementioned species overlap in Western Europe, forming a wide area of sympatry. The taiga tick I. persulcatus tends to inhabit patches of native coniferous and mixed taiga forests, whereas I. ricinus seems to prefer biotopes with the predominance of deciduous trees, as well as forest clearings. In the areas where these species overlap, there is a mixture of plant communities to which these species are confined. The biotopes of I. ricinus and I. persulcatus frequently coincide; it was ascertained that they may cohabit in Karelia and Tatarstan, as well as in the Leningradskaya, Pskovskaya and Vologodskaya Oblasts (Filippova 1977). In the northern and western areas of sympatry, the adults of *I. ricinus* and *I. persulcatus* may concurrently be trapped in the same biotopes (Korenberg *et al.* 1988). Immature stages (larvae and nymphs) of both species may frequently be found feeding on the same host species or even on the same host specimens of small mammals (Sukhomlynova 1977). The anthropogenic transformation of landscapes disrupts the natural biotopic association of ticks, leading to both species occurring in the same localities on the same hosts.

Interspecific hybridization is a common phenomenon in animal evolution. It is thought to eliminate the reproductive isolation between the parent species and can cause speciation. In nature, the interspecific hybridization becomes possible when the "original" species inhabit the same territory. The possibility of interspecific hybridization enhances the possibility that the representatives of both species will feed on the same host species, the same host individuals, and inhabit the same biotopes. This notion was proven by a laboratory crossing of ixodid ticks from the group of I. ricinus-I. persulcatus. The possibility of obtaining interspecific hybrids of I. ricinus and I. persulcatus, with a subsequent mortality of offspring during backcrossing, has been established earlier (Balashov *et al.* 1998a, b). The molecular-genetic investigations (Kovalev *et al.* 2016) have established the existence of genetic hybrids of these ixodid species among the natural populations of Estonia. The existence of 7–40% hybrids of *I. persulcatus* and *I. pavlovskyi* in their natural sympatric populations in Western Siberia has also been confirmed (Rar *et al.* 2019).

The first-generation hybrids with heterotic adaptability—by virtue of being part of natural ecosystems—may complicate the foci of natural infections. However, little is known about their role in natural ecosystems (Litov *et al.* 2022; Belova *et al.* 2023).

For a comprehensive investigation of hybrids in nature, it is necessary to study their diagnostic characters, which allow distinguishing them from each other and from the original species. The main purpose of our study is to investigate the external morphology of the immature stages of the European forest and taiga tick hybrids. Additionally, we examine the ecological characteristics of hybrids during their development and maintenance in natural habitats and natural inserts.

### MATERIALS AND METHODS

In this work, we used ticks from the laboratory culture maintained in Zoological Institute of the Russian Academy of Sciences, St. Petersburg, Russia (ZIN RAS). The ticks were collected in March-June 2000. Individuals of both sexes of *I. ricinus* were collected on the Curonian Spit, Kaliningradskaya Oblast, Rybachiy Village, 55°28'N, 20°58'E. Ixodes persulcatus were collected in the Leningradskaya Oblast, the Lava River, 59°50'N, 31°36'E. Ticks were kept at temperatures of 20-22 °C and at 96–98% humidity, with 16 light and 8 dark hours a day. The ticks were fed on rabbits. The fed nymphs were kept individually in glass tubes for the purposes of obtaining virgin females. Adult individuals, intended for crossbreeding, were united in parties of females and males, containing 10–15 individuals each (Table 1), which were kept together before and during feeding on different rabbits. The fed females were placed individually. This was done for the purposes of estimating the number of females, which laid eggs. We recorded the following parameters for each female: body weight after feeding, the number of eggs in the egg masses, the number of hatched larvae, as well as the number of hatched nymphs from each batch of fed larvae (50 individuals). In order to obtain nymphs, the hybrid larvae were fed for 1.5–2 months after hatching.

In order to identify reliable diagnostic characters, 10–15 individuals of hybrid larvae and nymphs from each egg mass were examined with a light dissecting microscope Levenhuk Zoom, with the magnification up to ×250 (Levenhuk Inc., Zoom&Joy, USA). The external morphology of larvae and nymphs of the hybrids of the European forest and taiga ticks were studied in detail with a SEM Quanta 250 (FEI Company, the Netherlands). In total, we have examined 90 larvae and nymphs of the hybrids F1  $\bigcirc$  *I. ricinus* ×  $\bigcirc$  *I. persulcatus* and 120 larvae and nymphs of the hybrids F1  $\bigcirc$  *I. persulcatus* ×  $\bigcirc$  *I. ricinus*.

The analysis of the diagnostic characters was performed with the help of the identification keys (Filippova 1977; Grigoryeva and Stanyukovich 2018).

To study the hybrids' ecological characteristics, the obtained egg masses, as well as the unfed and fully fed larvae and nymphs were kept in inserts, placed in natural biotopes according to procedures described previously (Grigoryeva and Stanyukovich 2016; Grigorieva and Shatrov 2022). It has been shown that this method allows establishing the peculiarities of the life cycle of hybrids (the duration of the larval and nymphal morphogenesis, the duration of their life span, etc.). In particular, the morphogenesis begins with the loss of mobility of the engorged larvae and nymphs and ends with the hatching of the next stage. At each developmental stage, the individual tick continues to develop after hatching, which does not prevent it from actively searching for the host, feeding and digesting the obtained blood. Only after the aforementioned activities, the next stage begins to form. During the season of tick activity, the changes in the quantities of ticks in the inserts were traced every 7-10 days.

## **RESULTS AND DISCUSSION**

As a result of hybridizing 6 of 8 engorged females of *I. ricinus* with the males of *I. persulcatus*, we have obtained the offspring F1  $\bigcirc$  *I. ricinus* ×  $\bigcirc$  *I. persulcatus*. Additionally, as a result of hybridizing 8 of 8 engorged females of *I. persulcatus* with the males of *I. ricinus*, we have obtained the offspring F1  $\bigcirc$  *I. persulcatus* ×  $\bigcirc$  *I. ricinus* (Table 1). The weight of the fed females was close to the average species value (Grigoryeva 2011), while the number of laid eggs was 2–3 times lower, when compared to intraspecific mating. The number of hatched larvae was 1.5–1.8 times lower than in the original species (Grigoryeva and Stanukovich 2016; Grigoryeva and Shatrov 2022). The number of eggs with an incomplete embryogenesis ranged from 36.5 to 45.7%, which is 4–8 times higher than in the parent species: *I. ricinus* and *I. persulcatus*. The proportion of hatched nymphs to the number of fed larvae was the same as during intraspecific mating, 85–89% (Grigoryeva and Stanukovich 2016; Grigoryeva and Shatrov 2022). The unfed and fully fed hybrid larvae and nymphs overwintered just as successful as did the individuals of the parent species (90-99%).

According to Kovalev et al. (2016), females of I. ricinus produce hybrids more successfully with the males of I. persulcatus than females of I. persulcatus with the males of I. ricinus. The same situation is found in reverse crossbreeding, i.e., when hybrids were crossing with the parent species. In this situation, females F1 preferably mate with I. ricinus males. However, in the earlier work on the hybridization of species from the group I. ricinus-I. persulcatus, the reverse situation was observed (Balashov et al. 1998a, b). In 10 pairs of female I. persulcatus-male I. ricinus, 7 females have laid eggs, whereas in 7 pairs of female I. ricinus-male I. persulcatus only 3 females laid eggs. Besides, the effectiveness of egg masses in the former pairs was higher than in the latter ones. The present study confirms and further strengthens the results obtained in previous investigations (Table 1).

The detailed study of the external morphology of the hybrid nymphs of I. ricinus and I. persulcatus has revealed a number of reliable diagnostic characters, which are important for the identification of hybrids and for distinguishing them from the parent species. It should be noted that, in nature, it is possible to collect larvae and nymphs mainly from the animals caught in Gero traps or live traps, which creates difficulties in their identification. Accurate identification is easier when working with unfed larvae and nymphs, or with the specimens that have just begun to feed (1 day of feeding). In fully fed individuals, the identification is difficult due to the increased body volume and the changes in the arrangement of the setae areas. In any case, the identification is based on comparing the length and the width of the main groups of setae on the dorsal and ventral body surfaces of the larvae and nymphs, with those in the diagnostic drawings (Fillipova 1977).

Nymphs F1  $\bigcirc$  *I. ricinus*  $\times \mathcal{J}$  *I. persulcatus.* On the dorsal side, the marginal setae of the alloscutum are well developed. They are located in the middle of the lateral roller, with 8-9 setae on each side of the alloscutum. These setae are long (Table 2), stout and overlapping each other. Premarginal setae are well developed; they form regular rows of 6-7 setae along the lateral bulges and correspond in size to the marginal setae. The middle setae (10-15) are well developed and are close in size to the marginal and premarginal setae. On the ventral side, among sternal, anterior epimeral and adanal setae of equal length and thickness, the posterior epimeral and adanal setae are prominent by their length; they are 1.5-2 times longer than others.

Nymphs F1  $\bigcirc$  *I. persulcatus*  $\times$   $\bigcirc$  *I. ricinus.* On the dorsal side, the marginal setae of the alloscutum, numbering 10–11, are well developed and are arranged in the same way as in the previous hybrids. They are long, stout and overlap each other. Premarginal setae, 5–6 on each side, and 10 middle setae are also long and stout. On the ventral side, all setae are sternal, with epimeral anterior, epimeral posterior, adanal anterior and adanal posterior approximately the same in length and width.

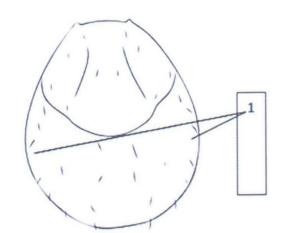
Nymphs *I. ricinus*. On the dorsal side, the marginal setae of the alloscutum are well developed. They are located in the middle of the lateral bulge, 11–12 setae on each side of the alloscutum. These setae are long and stout and overlap each other. They are close to premarginal and middle setae in size. On the ventral side, sternal, epimeral and adanal setae are quite similar in length and width.

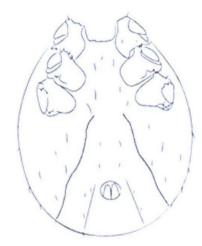
Nymphs *I. persulcatus*. On the dorsal side, the marginal setae of the alloscutum are well developed. They are located in the middle of the lateral bulge, 6–7 setae on each side of the alloscutum. They are also longer and thicker than the premarginal and middle setae. On the ventral side, sternal, epimeral and adanal setae are similar in length and thickness.

The original species and their hybrids differ in the number of marginal setae. Besides, in the nymphs of *I. persulcatus*, the posterior marginal setae do not apparently protrude beyond the posterior margin of the alloscutum, whereas in the nymphs of *I. ricinus* and their hybrids, these setae protrude significantly, sometimes by half of their length. In the original species, the sternal setae are slightly longer than the epimeral and adanal setae. In the hybrids F1 fem. *I. persulcatus* × male *I. ricinus*, the sizes of setae are small and similar. In the hybrids F1 fem. *I. ricinus* × male *I. persulcatus*, the size of the posterior epimeral and adanal setae exceeds that of the anterior setae by 1.5-2 times (Figs. 1–5).

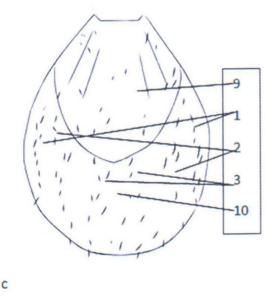
Recently, an attempt was made to present the morphometric characters of the hybrid nymphs of

*I. ricinus* and *I. persulcatus,* based on the sizes of the basic structures: scautum, capitulum, hypostome, palps, tarsus, coxae, scutal and alloscutal setae (Bugmyrin *et al.* 2016). However, the authors have failed to identify the accurate diagnostic characters that would allow to distinguish hybrids from the original species and from each other. The characters that we propose in this paper are more obvious. We were able to detect a stable





b



a

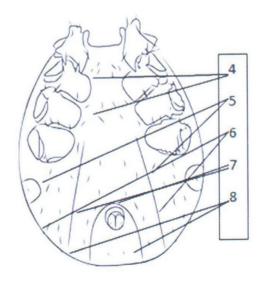


Fig. 1. Schematic drawings, illustrating the morphology of larvae (a—dorsal, b—ventral) and nymphs (c—dorsal, d—ventral) of ticks of the genus *Ixodes* (according to Filippova 1977). Setae: 1—marginal, 2—premarginal, 3—median, 4—sternal, 5—anterior epimeral, 6—posterior epimeral, 7—anterior adanal, 8—posterior adanal, 9—scutum, 10—alloscutum.

d

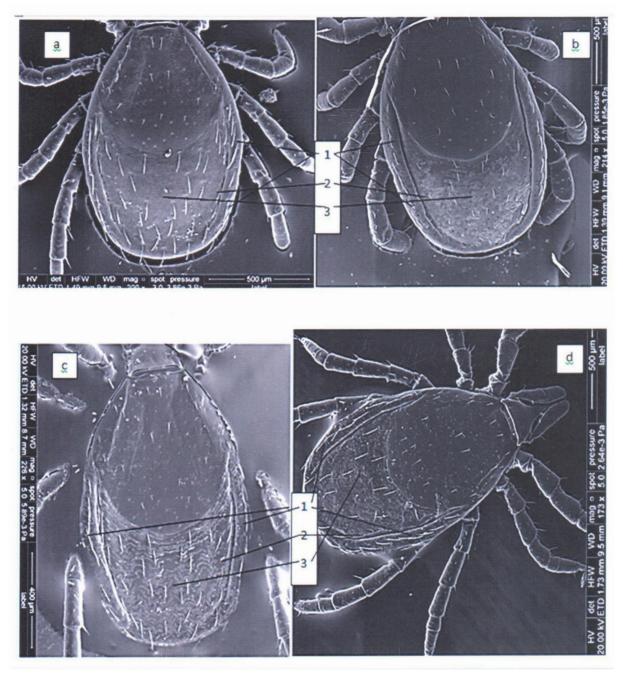


Fig. 2. Nymphs (a) *Ixodes ricinus*, (b) *I. persulcatus*, (c) F1  $\bigcirc$  *I. ricinus*  $\times$   $\bigcirc$  *I. persulcatus*, (d) F1  $\bigcirc$  *I. persulcatus*  $\times$   $\bigcirc$  *I. ricinus*. From the dorsal side. Setae designations as in Fig. 1.

repetition of the sizes of the main groups of setae on the dorsal and ventral sides of the idiosoma, which were clearly visible during the external examination at ×400.

The shape and size of the main external structures (scutum, capitulum, hypostome, palps, tarsus, coxa, scutal and alloscutal setae) are not very informative, when differentiating the larvae of sheep and taiga ticks from their hybrids. As shown previously (Grigoryeva and Stanukovich 2018), the larvae of *I. ricinus* are distinguished from those of *I. persulcatus* by well-developed posterior marginal setae of the alloscutum, which protrude beyond the posterior body margin by half of their length. However, the same characters are also characteristic of hybrids. Nevertheless, these characters may be discriminated by measuring the setae. The posterior marginal setae in the larvae of *I. ricinus* are  $0.043-0.05 \times 0.003-0.004$  mm, in *I. persulcatus*— $0.016-0.024 \times 0.004-0.005$  mm,

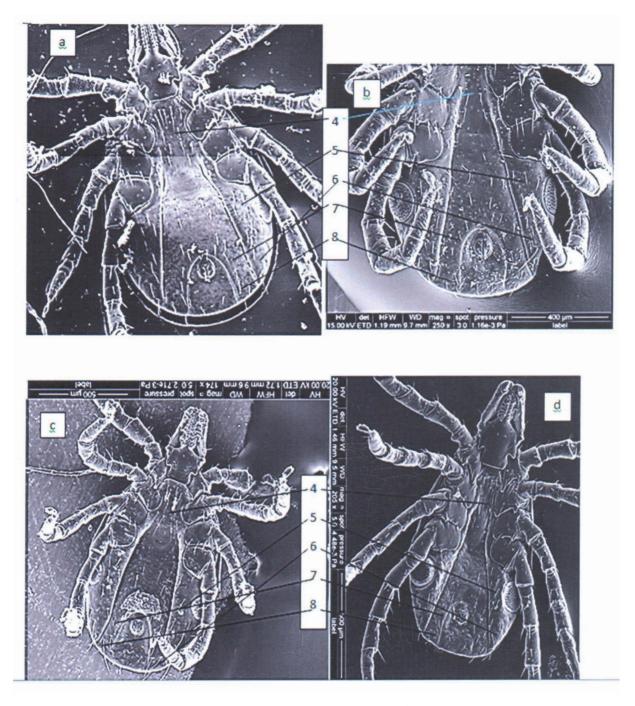


Fig. 3. Nymphs (a) *Ixodes ricinus*, (b) *I. persulcatus*, (c) F1  $\bigcirc$  *I. ricinus*  $\times$   $\bigcirc$  *I. persulcatus*, (d) F1  $\bigcirc$  *I. persulcatus*  $\times$   $\bigcirc$  *I. ricinus*. From the ventral side. Setae designations as in Fig. 1.

in F1  $\bigcirc$  *I. persulcatus*  $\times$   $\bigcirc$  *I. ricinus*—0.031–0.038  $\times$  0.005–0.006 mm, in F1  $\bigcirc$  *I. ricinus*  $\times$   $\bigcirc$  *I. persulcatus*—0.039–0.05  $\times$  0.006–0.007 mm (Figs. 4, 5). Unfortunately, besides sizes, there are no other reliable characters for distinguishing the larvae, as illustrated previously (Bugmyrin *et al.* 2015).

As noted above, the shapes and the sizes of the main morphological structures:—scutum, capitulum, hypostome, palps, palptarsus, coxae, scutal and alloscutal setae—are of little use when distinguishing the larvae and the nymphs of *I. ricinus* and *I. persulcatus* from their hybrids. Representatives of the same species from different populations exhibit wide and overlapping size ranges of the main structures, which can be attributed to individual variation (Filippova 1977). Nevertheless, chaetotaxy has long attracted the attention of taxonomists (Cliffors and Anastos 1960; Filippova

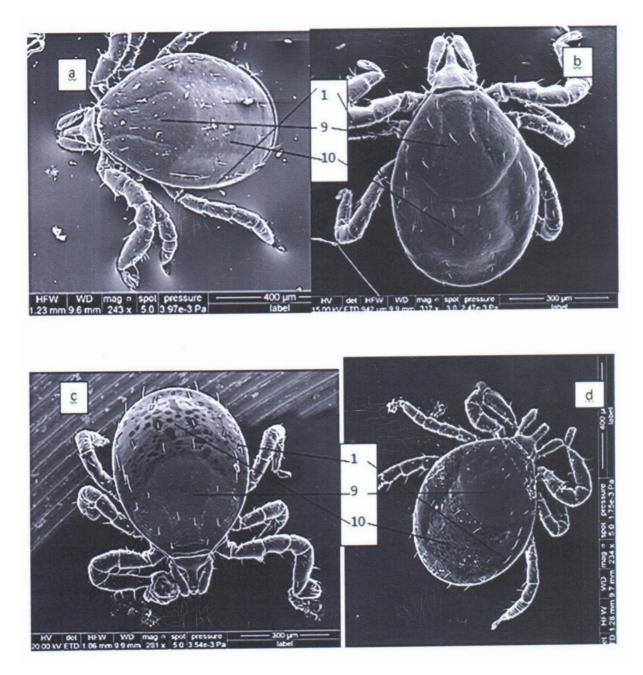
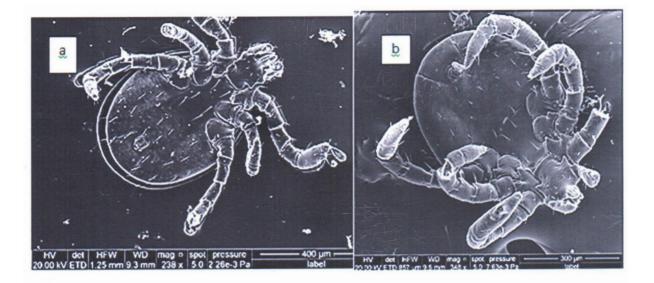


Fig. 4. Larvae (a) *Ixodes ricinus*, (b) *I. persulcatus*, (c) F1  $\bigcirc$  *I. ricinus*  $\times$   $\bigcirc$  *I. persulcatus*, (d) F1  $\bigcirc$  *I. persulcatus*  $\times$   $\bigcirc$  *I. ricinus*. From the dorsal side. Setae designations as in Fig. 1.

1977), and it is widely used in the systematics of ixodids, initially proposed by Filippova (1977). Besides systematical consideration, body setae have an important functional significance. Most of the setae covering the body function as tactile mechanoreceptors, whereas those located on the legs and the elements of the mouth apparatus function as contact chemoreceptors (Balashov 1998; Coons and Alberti 1999; Leonovich 2005). A detailed study of the ultrastructure of mechanoreceptors has shown that they transfer signals from the external sources to the central nervous system (Leonovich 1985). The above processes highlight their importance in the tick behavior. The tactile setae provide information about the physical properties of the substrate during the tick's movement through the soil litter. This helps the tick orientate successfully in the surrounding environment. Observations of ticks in natural inserts indicate that the idiosomal setae may help increase the air volume around the body. This may prevent the ticks from adhering to wet substrates, which is important



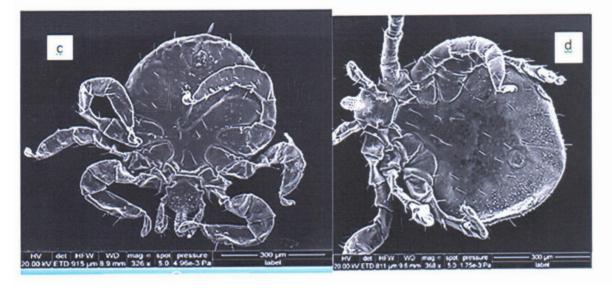


Fig. 5. Larvae (a) *Ixodes ricinus*, (b) *I. persulcatus*, (c) F1  $\stackrel{\frown}{\rightarrow}$  *I. ricinus* ×  $\stackrel{\circ}{\rightarrow}$  *I. persulcatus*, (d) F1  $\stackrel{\bigcirc}{\rightarrow}$  *I. persulcatus* ×  $\stackrel{\circ}{\rightarrow}$  *I. ricinus*. From the ventral side.

for ticks during their movements through the leaves in soil. Body setae also seem to facilitate the tick's movement through the host animal's hair in the course of attacking and feeding. Probably, body setae also facilitate the interaction of sexes during mating (Grigoryeva unpubl. data).

The observations of the life and development cycle of hybrid larvae and nymphs in natural inserts have shown that the duration of the period from the beginning of feeding of a female to the beginning of the oviposition was around 20–35 days (it is known that the initial stages of oogenesis in ixodids occur even before feeding (Balashov 1998)). The lifespan of larvae F1  $\bigcirc$  *I. persulcatus* ×  $\bigcirc$  *I. ricinus* is 14–16 months, and of larvae F1

 $\bigcirc$  *I. ricinus*  $\times$   $\bigcirc$  *I. persulcatus*—12–15 months. The lifespan of nymphs F1  $\bigcirc$  *I. persulcatus*  $\times$   $\bigcirc$  *I. ricinus* is 14–16 months as well, and of nymphs F1  $\bigcirc$  *I. ricinus*  $\times$   $\bigcirc$  *I. persulcatus*—12–15 months.

Considering that the individuals of the parent species may mate in nature when their seasons of activity overlap (from late April until July), the hybrid larvae may appear in July–August of the same year. The duration of the larvae morphogenesis was around 30–40 days in both hybrids. Unfed larvae F1  $\bigcirc$  *I. ricinus*  $\times \bigcirc$  *I. persulcatus* can survive up to July–October, and the larvae of F1  $\bigcirc$  *I. persulcatus*  $\times \bigcirc$  *I. ricinus*—up to September–November of the next year. In the course of feeding of larvae during the season of active hatching or during the

first half of summer of the next year, the nymphal morphogenesis with a subsequent molting into nymphs completes in July-August of the second year of the life cycle. In F1  $\bigcirc$  *I. ricinus*  $\times \bigcirc$  *I. per*sulcatus this process lasts 30-40 days, without wintering. The period of wintering with a diapause and with the digestion of blood has prolonged the time of the nymphal hatching to 10-12 months. In the fed larvae F1  $\bigcirc$  *I. persulcatus*  $\times \bigcirc$  *I. ricinus*, the nymphal morphogenesis lasted 40-48 days; however, with a diapause, the hatching of the nymphs occurred only in 10-12 months. Consequently, when accounting for the diapause, the life cycle has increased to a year. The development patterns of the preimaginal phases of the I. ricinus and I. persulcatus hybrids are similar (Table 3).

The phenomenon of the cohabitation of ticks I. ricinus and I. persulcatus and the possibility of their hybridization is important for understanding the role of sympatry in the dynamics of parasitic systems. When it comes to sympatry, species display a non-absolute biotopic differentiation. The parasitism/feeding seasons of the pre-imaginal life-cycle stages of these species and their hybrids mostly coincide (Grigoryeva et al. 2019). Immature stages of both species occur on the same host species and sometimes on the same host individuals (small mammals). Such sympatric relationships indicate the presence of evolutionary established prerequisites for the alternate entry of a pathogen into the bodies of both tick species. Close affinity of the tick species allows the pathogen to successfully survive and develop. The possibility of the horizontal and vertical circulations of pathogens in many sympatric generations may increase the intensity of the pathogen exchange. Consequently, in the natural foci of the same infection, the presence of taiga and sheep ticks as well as their hybrids, may lead to differences in the infection foci's manifestation in each particular case. Therefore, pathogen circulation patterns may differ significantly in the areas of separate and joint habitats of closely related vector species. The particulars of the pathogens and the characteristics of their relationships with the vectors may be associated with the sympatry of the vector species and the increased adaptability of their hybrids. For example, the lifespan of the hybrid species is greater than that of the parent species, which is 11-12 and 10-11 months for the larvae and the nymphs of I. persulcatus, respectively (Grigoryeva and Stanyukovich 2016).

#### CONCLUSION

The presented comparative diagnosis of the preimaginal phases of the hybrids of the European forest and taiga ticks (and their differences from the original species) are based on the relative size and quantity of the marginal, premarginal, and median setae on the dorsal side, as well as on the relative size and quantity of the sternal, epimeral, and adanal setae on the ventral side in nymphs. The examined species and their hybrids differ in the quantity of marginal setae. In particular, in the nymphs of *I. persulcatus*, the posterior marginal setae are just barely prominent beyond the posterior edge of the alloscutum, whereas in the nymphs of I. ricinus and their hybrids, they are significantly prominent, sometimes by half their length. In the parent species, the sternal setae are slightly longer than the epimeral and adanal setae. In the hybrids F1  $\bigcirc$  *I. persulcatus*  $\times \bigcirc$  *I. ricinus*, the sizes of these setae are very small and similar to each other. In the hybrids F1  $\bigcirc$  *I. ricinus*  $\times \stackrel{\sim}{\supset} I$ . *per*sulcatus, the posterior epimeral and adanal setae exceed the anterior setae by 1.5-2 times. The diagnosis of the hybrid larvae is difficult; they can be distinguished based on the morphometry of the marginal setae. The posterior marginal setae in the larvae of I. ricinus are  $0.043-0.05 \times 0.003-$ 0.004 mm, in the larvae of I. persulca*tus*— $0.016-0.024 \times 0.004-0.005$  mm, in the larvae of F1  $\bigcirc$  *I. persulcatus*  $\times$   $\bigcirc$  *I. ricinus*—0.031–0.038  $\times$ 0.005–0.006 mm, in the larvae of F1  $\bigcirc$  *I. ricinus* × 3 *I. persulcatus*—0.039–0.05 × 0.006–0.007 mm. Apart from the size ranges of the marginal setae of the alloscutum in the larvae, it is not possible to identify other characters.

Hybrid larvae may hatch in July-August of the year that the parental individuals mated. The duration of the larval morphogenesis is 30-40 days for both hybrids. The unfed/hungry larvae can survive until July–October (F1  $\bigcirc$  *I. ricinus*  $\times \bigcirc$  *I. persulcatus*) and September–November (F1  $\bigcirc$  *I. persulcatus*  $\times \stackrel{\sim}{\mathcal{O}}$  *I. ricinus*) of the next year. If the larvae feed in the year of hatching or in the first half of the next year's summer, the nymphal morphogenesis with the following molting into nymphs completes in July2August of the second year and, in F1  $\bigcirc$  *I. ricinus*  $\times$   $\bigcirc$  *I. persulcatus*, lasts 30–40 days without wintering. When accounting for the diapause, the period of wintering and blood digestion increases the nymphs' time to hatching by another 10–12 months. In F1  $\bigcirc$  *I. persulcatus* ×  $\bigcirc$  *I. ricinus*, the nymphal morphogenesis lasts 40-48 days, and with a diapause, the hatching of the nymphs occurs in 10–12 months. The lifespan of the unfed larvae of F1  $\bigcirc$  *I. persulcatus*  $\times \bigcirc$  *I. ricinus* is 14–16 months, and of the unfed larvae of F1  $\bigcirc$  *I. ricinus*  $\times$  $\oslash$  *I. persulcatus*—12–15 months. The lifespan of the unfed nymphs of F1  $\bigcirc$  *I. persulcatus*  $\times \bigcirc$  *I. ricinus* is 14–16 months, whereas that of the unfed nymphs of F1  $\bigcirc$  *I. ricinus*  $\times \bigcirc$  *I. persulcatus*—12–15 months.

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#### L.A. Grigoryeva et al.

Species and the no. of ticks in mating	females	10 <i>Ip</i>	10 <i>Ir</i>
	males	15 Ir	15 Ip
No. of females	attached	10	10
	engorged	9	8
Weight of engorged females (avg, minmax.), mg		358.3 (208.1–434.7)	387.2 (246.2–443.5)
No. of females laying eggs		8	6
No. of eggs, total and laid by one female (min.–max.)		5,687 (275–1,020)	1,035 (154–508)
No. of fertile clutches		8	6
No. of hatched larvae, total, in one clutch (min.–max.)		3087 (287–513)	657 (47–186)

Table 1. The results of the interspecies crossbreeding of I. persulcatus (Ip) and I. ricinus (Ir).

Table 2. The morphometric characteristics of the diagnostic structures of the nymphs of *I. ricinus*, *I. persulcatus* and their hybrids (μm).

Name of the structure, setae	I. ricinus	I. persulcatus	F1 $\bigcirc$ <i>I. ricinus</i> × $\bigcirc$ <i>I. persulcatus</i>	F1 $\bigcirc$ <i>I. persulcatus</i> × $\bigcirc$ <i>I. ricinus</i>
marginal epimeral and adanal	78.6±2.9 × 8.5±0.2	46.1±0.31 × 6.5±0.18	78.2±1.69 × 7.0±0.44	80.3±1.6 × 5.5±0.18
premarginal	$80.1 \pm 1.6 \times 8.5 \pm 0.2$	31.5±0.21 × 4.5±0.19	$78.3 \pm 1.9 \times 7.0 \pm 0.44$	$72.0\pm0.28 \times 4.5\pm0.19$
median	$80.1 \pm 1.6 \times 8.5 \pm 0.2$	$31.5\pm0.21 \times 4.0\pm0.19$	$60.5 \pm 1.71 \times 7.1 \pm 0.44$	$58.5 \pm 1.35 \times 4.5 \pm 0.19$
sternal	$51.0\pm0.26 \times 5.5\pm0.18$	$52.2 \pm 0.88 \times 4.5 \pm 0.19$	$62.1 \pm 0.28 \times 6.5 \pm 0.18$	$43.5 \pm 1.90 \times 4.5 \pm 0.19$
epimeral anterior	$9.5 \pm 1.34 \times 7.4 \pm 0.36$	$40.5\pm0.80 \times 4.5\pm0.19$	62.0±0.28 × 6.5±0.18	37.1±1.03 × 4.5±0.19
epimeral posterior	$9.5 \pm 1.34 \times 7.4 \pm 0.36$	$40.5\pm0.80 \times 4.5\pm0.19$	90.0±2.06 × 10.5±0.21	37.0±1.03 × 4.5±0.19
adanal anterior	$39.5 \pm 1.34 \times 7.4 \pm 0.36$	33.2±1.21 × 4.5±0.19	$55.1 \pm 1.12 \times 5.5 \pm 0.18$	$37.0\pm1.03 \times 4.5\pm0.19$
adanal posterior	39.5±1.34 × 7.4±0.36	33.2±1.21 × 4.5±0.19	90.1±2.06 × 6.5±0.18	37.0±1.03 × 4.5±0.19

Table 3. The sequence of the developmental stages of the hybrid larvae and the nymphs of F1  $\bigcirc$  *I. persulcatus* ×  $\bigcirc$  *I. ricinus* and F1  $\bigcirc$  *I. ricinus* ×  $\bigcirc$  *I. persulcatus* in natural inserts.

1.	F1 $\bigcirc$ <i>I. persulcatus</i> $\times \stackrel{\circ}{\supset} I.$ <i>ricinus</i> F1 $\bigcirc$ <i>I. ricinus</i> $\times \stackrel{\circ}{\supset} I.$ <i>persulcatus</i>				
2.	Nutrition of female parent pairs April–June, 1 <sup>st</sup> year				
3.	Egg laying May–July, 1 <sup>st</sup> year				
4.	Larval hatching August–September, 1st year				
5.	Larval feeding September–October, 1 <sup>st</sup> year, May–July, 2 <sup>nd</sup> year	Larval feeding July–October, 2 <sup>nd</sup> year			
6.	Nymphal hatching August–September, 2 <sup>nd</sup> year	Nymphal hatching August–September, 3 <sup>rd</sup> year			
7.	Nymphal feeding September–October, 2 <sup>nd</sup> year, May–July, 3 <sup>rd</sup> year	Nymphal feeding July– October, 3 <sup>rd</sup> year, May– July, 4 <sup>th</sup> year	Nymphal feeding August– October, 4 <sup>th</sup> year		