Palaeoclimatic models - predicted changes in the potential Neogene distribution patterns of *Phlebotomus similis* and *Phlebotomus sergenti* (Insecta: Diptera: Psychodidae)

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Abstract

Former studies proposed that the speciation of the subgenus *Paraphlebotomus* happened in the Neogene Epoch in the circum-Mediterranean region due to the geographical segregation effect of the former Paratethys Sea. It was aimed to study whether the modelled Neogene ranges of *Phlebotomus sergenti* and *Phlebotomus similis* support or contradict this barrier role of the Paratethys in the speciation of *Paraphlebotomus* sandfly. For this purpose, the potential Neogene geographical ranges of *Phlebotomus sergenti* and *Phlebotomus similis* were modelled based on the present climatic requirements of the taxa. The Miocene models do not support the circum-Paratethyan migration of the ancestor of *Phlebotomus similis*. In general, *Phlebotomus similis* shows a low affinity to the North Paratethyan shorelines during the entire Miocene epoch. The only exceptions are the Tortonian and early Messinian periods when the climatic conditions could be suitable for *Phlebotomus similis* in the North Paratethyan shorelines. It was found that neither the modelled late Miocene, Pliocene nor the mid-Pleistocene period distributions of *Phlebotomus sergenti* and *Phlebotomus similis* shows notable differences in the suitability values in the Balkans and the Middle East. It is most plausible that the divergence of the *Phlebotomus similis* and its relatives was related to the tectonic subsidence of the Hellenic Orogenic Belt and *Phlebotomus similis* specialised in the Balkan Peninsula and the present-day North Pontic area during the middle-late Miocene epoch. The Messinian desiccations of the Mediterranean Basin and the Zanclean re-flood caused the migration, but not the speciation of *Phlebotomus similis* and its sister taxa.

Keywords Palaeoecology · Palaeobiogeography · *Phlebotomus* · Divergence · Ecological modelling

Introduction

As members of the Mediterranean fauna, the Mediterranean sandfly species are the vectors of several Phleboviruses and *Leishmania* parasites which are the causative agents of serious veterinary and human diseases. Leishmaniasis is an emerging epidemiological hazard in Europe (Ready 2010). Sandfly-borne diseases not only mean significant health risks, but they also impose a notable economic burden on society. For example, only in Italy, the direct costs for hospital admissions related to human leishmaniasis were between 1,637 and 1,370 thousand euro per year in the period 1999–2003 (Mannocci et al. 2007). Sandfly-borne diseases also cause great veterinary expenditures in Europe. In Spain, a study revealed that up to 13–20% of dogs

can be infected with *Leishmania infantum* Nicolle, 1908 in the thermo-Mediterranean and meso-Mediterranean territories (Martín-Sánchez et al. 2009). These dogs also serve as the source of zoonotic leishmaniasis for humans (Quinnell and Courtenay 2009). The members of the Phlebotomine sandfly subgenus *Paraphlebotomus* are typical members of the Mediterranean entomofauna. *Phlebotomus* (*Paraphlebotomus*) *sergenti* Parrot, 1917, the main and only proven vector of *Leishmania tropica* Wright, 1903 among the members of the subgenus *Paraphlebotomus* (Depaquit et al. 2002), presently has a circum-Mediterranean range, including North Africa, the Hispanian Peninsula, South France, Sicily, the Balkans, the Greek islands, Asia Minor, Levant and the Caucasus (ECDC 2020). In contrast, *Phlebotomus* (*Paraphlebotomus*) *similis* Perfiliev, 1963, the second, suspected vector of *L. tropica* within the subgenus *Paraphlebotomus* (Antonioni et al. 2013), only can be found in the Balkans, the Greek islands and Asia Minor (ECDC 2020).

There is growing interest to understand the influences of past climate changes on vector arthropod species for the better

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prediction of the possible effects of the anthropogenic climate change (e.g. Perrotey et al. 2005). It seems that the Neogene period played an important role in the speciation and the determination of the present climatic requirements of certain members of the Mediterranean arthropod fauna (e.g. Essegir et al. 1997, 2000; Depaquit et al. 2000; Trájer et al. 2018a). The Neogene palaeogeography of the Tethys and the Paratethys and the Pliocene-Pleistocene climatic fluctuations strongly influenced the recent area and the primary diversification of several circum-Mediterranean faunas (Oosterbroek and Arntzen 1992). This is rooted in the parallel cooling of the Earth from the turn of the Oligocene and Miocene epochs (McKay et al. 2016) and the complex geographical history of the circum-Mediterranean area which was strongly affected by the tectonic motions of Africa, Europe and several minor landmasses during the Alpine orogenesis (Rosenbaum et al. 2002). The opening of the Provencal Basin in the Oligocene, and the extension of the Pannonian Basin during the Miocene, fundamentally transformed the geography of the entire Mediterranean area (Carminati and Doglioni 2005; Balázs et al. 2016). In the early Miocene epoch (ca. 20 Ma), the Paratethys was well-connected with the Mediterranean (the Tethys) Sea and the Indian Ocean (Steininger and Rögl 1984). The geography of the seaways and the level of connectivity between the two large seas dramatically changed in the Neogene Epoch (Popov et al. 2006). In the middle Miocene era, the Dinaric-Hellenic Orogenic Arc formed a land bridge between Asia and the land of the present-day Balkan Peninsula (Jones et al. 1992). At that time, the fauna of the Balkan and the Middle East was separated from continental Europe due to the Central Paratethys Basin and the Slovenian corridor (Fodor et al. 1998). In the early Serravallian (ca. 13 Ma), the waters of the Central Paratethys and the Mediterranean Sea communicated with each other (Bartol et al. 2014) which caused the alternating level of separation of the landmasses of the Central Paratethys, the Middle East and the European Continent. In the latest Miocene period, the Messinian, the Tethys repeatedly desiccated and flooded again, which allowed the terrestrial fauna to migrate to the islands of the Mediterranean (Hsü 1974). Generally, this event is thought to be a milestone in the speciation of the Mediterranean sandfly species (e.g. Kasap et al. 2015). In the Pliocene epoch, the former Paratethys Sea was divided into several inland seas, which meant the disappearance of the former geographical barrier between the Middle East and Europe (Popov et al. 2006) (Fig. 1).

It is very plausible that the origin of the different actual ranges of the species goes back at least to the first part of the Neogene epoch (Essegir et al. 2000; Trájer et al. 2018a). The colonisation of the Mediterranean Basin by the subgenus *Paraphlebotomus* may have occurred in the Miocene period (Depaquit et al. 1998). The extant Mediterranean *Paraphlebotomus* species had a common Asiatic ancestor and the centre of dispersion of the genus

Paraphlebotomus might have been somewhere in South Asia (Depaquit et al. 2000; Alten 2010). Two migration routes were proposed, one, north of the Paratethys Sea and another south of the Paratethys and Tethys Seas. The first route was postulated as the cause for the isolation of *Ph. similis*, the second route may have contributed to the speciation of *Ph. sergenti* (Depaquit et al. 1998). Based on the internal transcribed spacer 2 (ITS2) sequences of 12 sandfly populations from 10 countries, Depaquit et al. (2002) and Alten (2010) found that the ancestor of *Ph. similis* originally colonised the Caucasus, the Crimean and the Balkan Peninsulas, including the Aegean islands and the western part of Asia Minor. In contrast, it was hypothesised that *Ph. sergenti* followed an east-west Asiatic migration route through the landmasses between the former Tethys and the Paratethys Seas in Asia. The species colonised North Africa and migrated to the Iberian Peninsula passing through the formerly dryland Betic Bridge (Léger and Depaquit 2002; Alten 2010). Essegir et al. (2000) and Trájer et al. (2018a) found that the ecological niches and the climatic distribution limiting values of the extant Mediterranean *Phlebotomus* species are the consequence of the climatic and geographical events of the Neogene era. While the present eastern distribution area of *Ph. sergenti* and *Ph. similis* is highly similar, the distribution limiting values of the two species are somewhat different. This may be due to the fact that the two species developed in different areas and climate zones (Trájer et al. 2018a). Based on the proposed North Paratethyan former range of *Ph. similis*, it can be hypothesised that this species should be more cold-tolerant than *Ph. sergenti*, which might rather have kept the climatic needs of the common ancestor of the two taxa. Because the retreat of the Central Paratethys Sea occurred in the Pliocene Period, this era may be the time when the geographical barrier between the two species disappeared. If the zoogeographic hypothesis outlined above is true, the modelled distributions of the ancestors of the two species had to be different around the Paratethys realm in the Pliocene era.

Aims

It was aimed to test the hypothesis that *Ph. similis* originally evolved in the northern, *Ph. sergenti* on the southern coastal areas of the Paratethys. To investigate the hypothesis, it was aimed to model the middle Pliocene and middle Pleistocene potential distribution of *Ph. similis* and *Ph. sergenti*, the climatic suitability of the North Pontic area for *Ph. similis* during the Miocene epoch and the suitability of the East Mediterranean and Paratethys area for the two species in the late Miocene (Tortonian) era. The approach was based on the principle of actualism, using the present climatic requirements of the species for Europe and the Middle Paratethys region. A specific aim was to examine whether *Ph. similis* could have more affinity to the North Paratethys region than its sister taxa or not. If the proposed difference in the potential ranges cannot be confirmed, the modification of the

Fig. 1 The geography of the wider Mediterranean area in the Sarmatian (late Serravallian), ca. 13–14 Ma

speciation theory of the two species in correspondence with the previous phylogenetic results of the literature is required.

Materials and methods

The logical framework and methodological considerations of the study

The following work plan has been identified:

- 1) According to the previously published *Paraphlebotomus* speciation theories, the North Pontic area, as the part of the northern coastline region of the East Paratethys Basin, should have a prominent role in the speciation of *Ph. similis* (Dapaquit 1998, 2000, 2002). Due to geographical considerations, this area could also be the source of the southward migration of the species further to their current in Southeast Europe and Asia Minor in the Pliocene, when the remains of the Paratethys became individual seas. Due to these considerations, the suitability of the North Pontic area was modelled for the entire Miocene epoch to determine whether this area could be the speciation centre and migration route of the species or not (model A).

- 2) Because the Tortonian-early Messinian era could be the last period when the speciation of *Paraphlebotomus* occurred (see the ‘Discussion’ section for the explanation), this period which is called ‘Pannonian’ in the Central Paratethyan regional stratigraphy was considered separately in the modelling (model B).
- 3) The mid-Pliocene and mid-Pleistocene potential distribution of *Ph. sergenti* and *Ph. similis* were modelled to investigate the similarities and differences of the distribution of the two species around the Central and East Paratethys area, after the disintegration into sub-basins of the formerly unified Paratethys Sea. This step is important to investigate whether immediately after the Pliocene disappearance of the unified basin *Ph. similis* had a better climatic affinity to the North Paratethys area compared to *Ph. sergenti* or not because this time was the time when the geographical segregation of the *Ph. similis* and its sister taxa ended (model C).

It is a basic methodological difficulty that if we look back to the ages of earth history, we have no statistically significant amount of occurrence data which could provide the basis for the determination of distribution limiting climatic values. Fossil Phlebotominae species can be found in the fossil record, e.g. †*Phlebotomites aphoe* Stebner et al., 2015, †*Phlebotomites*

burmatus Malak et al., 2013, †*Phlebotomites grimaldii* Malak et al., 2013, †*Phlebotomus khludae* Stebner et al., 2015, †*Phlebotomites neli* Malak et al., 2013 and †*Phlebotomus vetus* Stebner et al., 2015 from the Burmese amber (Stebner et al. 2015), which is in a Cenomanian (99.7 to 94.3 Ma old) terrestrial amber in Myanmar or †*Phlebotomus pungens* Loew, 1845 from a single Quaternary occurrence of Tanzania (Ross 2018). This fossil record is so poor that the known occurrence data of extinct species is completely useless for modelling purposes. Naturally, it cannot be set that the ecological (e.g. climatic) requirements of the ancestors of the extant species did not change in the Neogene period and were equal to the ecological demands of the present species. Although the environmental requirements of the ancestors of the recent sandfly species are not known, the climatic demands of the extant species can provide the basis for the examination of the Miocene-Pliocene-Middle Pleistocene distribution of the taxa. Climate determines the range of sandfly taxa, and it is possible that climatic variables also strongly influenced the past occurrences of the species. As it was described in the ‘Introduction’, sandfly species appear to be quite conservative in their environmental demands, and it is possible that the environmental requirements of the Mediterranean sandfly were determined by the climatic effects and geographic conditions of the Neogene period (Esseghir et al. 2000; Trájer et al. 2018a). It is also known that the distribution limiting climatic values of *Ph. sergenti*, which is one of the closest living relatives of *Ph. similis*, is very similar to the previous species (Table 1; Trájer et al. 2013) even though the divergence of the two species should occur in the mid-Miocene epoch, before the disappearance of the Paratethys Sea (Depaquit et al. 2002). This indicates that the common ancestors of the two species may have had similar climatic needs, and that, throughout their history, similar climatic needs have been maintained by the sister species. Based on the above-described considerations, although with certain restrictions and cautions, the present climatic requirements of the two species can be used as the basis of the modelling of the former distribution of their ancestors.

Although *Phlebotomus* (*Paraphlebotomus*) *jacusieli* Theodor, 1947 has a slightly closer phylogenetic relationship

to *Ph. similis* than *Ph. sergenti* (Depaquit et al. 2002), the past occurrences of *Ph. jacusieli* were not modelled in the present study. This is partly because of the previously established speciation models, which described the parallel development of *Ph. similis* and *Ph. sergenti*. On the other hand, the climatic requirements of *Ph. jacusieli* are not known sufficiently. For these reasons, in this study, primarily the speciation of *Ph. similis* in the Miocene epoch, and in parallel, the potential former distribution of *Ph. sergenti*, was studied in the mid-Pliocene and the mid-Pleistocene periods.

The climatic extrema used in the study

To create the distribution models, the occurrence limiting climatic variables of Trájer et al. (2013) were used. The authors performed their analyses based on the distribution data of the European Centre for Disease Prevention and Control. This uses several occurrence categories, like ‘present’ and ‘introduced’. Trájer et al. (2013) handled only the ‘present’ category data as positive areas for permanent occurrence. For the explanation of the employed distribution limiting values, see the model identification subchapters.

Sources of the used climate models

The Miocene climate models used in this study were based on the determination of the differences between the past and the present bioclimatic values. These regional climate models were created by the modification of the reference period’s climate model using the difference values from the present climatic conditions in the case of each climatic factor. To maintain the validity and reliability of the developed models, the georeferenced models were only specified for the northern area of the Black Sea. The palaeoclimatic data of the North Pontic region was gained from Syabryaj et al. (2007). Because the reconstructed values were not directly used in modelling and the utilisation of climatic reconstructions for modelling purposes cannot be distinguished from the explanation of the development of *model environment A*, see the detailed

Table 1 The ages of the fossil palaeoflora sites in the palaeoclimatic reconstruction of Syabryaj et al. (2007) expressed in regional and global stratigraphic units; *local, southern Ukrainian stratigraphic unit, **Paratethyan stratigraphic unit, ***global stratigraphic unit

Ages in the original units	Ages in global units	Start-end (approx., Ma)	Fossil site; no. of the strata	Long. (E); lat. (N)
Aquitania***	Aquitania	23.0–20.4	Bolshie Kopani 1	32.97; 46.48
Burdigalian***	Burdigalian	20.3–16.0	Bolshie Kopani 2	32.97; 46.48
Tarkhanian*	Early-middle Langhian	16.2–14.9	Leninskoe	34.03; 45.27
Badenian **	Langhian and Ealy Serravallian	16.3–12.8	Korobki 1,2,3	33.43; 45.27
Sarmatian**	Late Serravallian	12.7–11.6	Emetovka 1	30.52; 46.75
Maeotian*	Late Tortonian-Early Messinian	9.0–6.3	Emetovka 2,3	30.52; 46.75
Pontian**	Late Messinian	6.2–5.3	Chaplinka	33.53; 46.37

explanation of this climate model in the related subchapter ‘Model identification of model environment A’. The *model environment B* covers the Tortonian period, which is equal to the Paratethyan regional stratigraphy unit Pannonian period *sensu stricto*. The Pannonian (Tortonian) climate model was based on the palaeoclimatic reconstruction of Bruch et al. (2006). In *model environment C*, previously developed, global-scale climate models were used. The georeferenced climate data were taken from WorldClim database. The WorldClim version 2 (Fick and Hijmans 2017) has average monthly climate data for minimum, mean and maximum temperature and for precipitation for 1970–2000, which reference period was used for comparison and verification purposes. For modelling the past conditions, cold and warm period models of the mid-Pliocene epoch were used. The cold period model was constructed by Dolan et al. (2015) and it represents the M2 mid-Pliocene warm period 3.264–3.025 Ma. The warm period model was constructed by Hill (2015) and represents the middle Pliocene glaciation stage 3.3 Ma (more exactly: 3.29–3.42 Ma) in the Piacenzian. The MIS19 mid-Pleistocene period’s model (ca. 787 ka) was constructed by Brown et al. (2018).

Topographical reconstructions

Naturally, the sea level model of the Miocene epoch can only be indicative. It cannot be the object of a palaeobiogeographic study to exactly reconstruct the past fluctuations of the global and the Paratethyan sea levels or the tectonic history of the area. What is important in this aspect is that the Mediterranean terranes approximately reached their present positions at the turn of the Oligocene and Miocene epochs in the Central Paratethys (Kvaček et al. 2006; Kováč et al. 2017) creating geographic patterns that were similar to the present conditions in the central basins of the sea. For a general approximation, it can be accepted that about a plus 60-m enhancement of the present sea level conditions can be used to indicate the former seashore patterns. The geological reconstructions show very similar shorelines in the Paratethys that was used in the present study (compare with the reconstructions of Popov et al. 2006; Palcu et al. 2019). The approximate shoreline patterns of the Pannonian Lake in about 10 Ma in model C was based on Magyar et al. (1999).

Identification of model environment A

The first model environment (*model environment A*) was performed to model the Miocene potential distribution of *Ph. similis*, using the climatic requirements of the extant species. Since available, high-resolution climate models were not available for the North Pontic area, which is identical to the former northwestern seashore region of the East Paratethys, climate models were developed based on the modifications

of the reference climate maps according to the palaeoclimatic data of Syabryaj et al. (2007). The authors investigated the fossil Miocene vegetation of South Ukraine. Because the Miocene strata of Ukraine belong to the Central and Eastern Paratethys, this region is the target area of the speciation of *Ph. similis* according to the theory of Depaquit (1998) and Alten (2010). Using the Coexistence Approach (Mosbrugger and Utescher 1997), Syabryaj et al. (2007) reconstructed the Miocene palaeoclimate of the region for the Aquitanian, Burdigalian, Tarkhanian (lower-middle Langhian), Badenian (Langhian and lower Serravallian), Sarmatian (upper Serravallian), Maeotian (upper Tortonian-lower Messinian) and the Pontian (upper Messinian) ages (for the explanation of the periods see Table 1). The reconstruction of Syabryaj et al. (2007) does not cover the period of the Pannonian regional stratigraphic unit (the Tortonian).

The Coexistence Approach concept is based on the distribution of limiting values of the closest extant relatives of the fossil taxa. This method can be accepted for the Neogene period due to the time proximity; if the fossil record is treated with caution, many fossil taxa are involved, and the climatic demands of the living taxa are analysed with appropriate statistical tools (Mosbrugger and Utescher 1997). Syabryaj et al. (2007) investigated microflora and concerned three thermal and four precipitation climate variables: the mean annual temperature, the cold month mean temperature, the warm month mean temperature, the mean annual precipitation, the precipitation in the warmest month, the precipitation in the driest month and the precipitation in the wettest month. Because Syabryaj et al. (2007) used classic bioclimatic categories, some georeferenced bioclimatic variables of the PaleoClim database could easily correspond, others not. To increase the number of variables, two derived variables, i.e. the annual monthly average and July Thornthwaite agrometeorological aridity indices (sing. abbr.: TAI), were introduced which values directly can be derived from the precipitation and temperature values (Kemp, 2002). The common formula of the TAI index is the following:

$$\text{TAI} = 1.65 \times \frac{P}{T + 12.2} \left(\frac{10}{9} \right)$$

where TAI is the Thornthwaite agrometeorological aridity index in millimetres per °C, P is the monthly precipitation sum in millimetres and T is the monthly mean temperature in °C.

In the present study, the annual mean values of the climatic variables and the July values were used. The use of the aridity index is justified by the fact that sandfly species are highly sensitive to the combination of strong solar radiation and atmospheric drought (El-naïem et al. 1998; Trájer et al. 2018b).

The used climatic variables were as follows: the lower and upper limits of the annual mean of the temperature (T_{am} ; °C;

Bioclim1), the lower and upper limits of the January mean temperature ($T1min,max$, °C); the lower and upper limits of the July mean temperature ($T7min,max$, °C); the lower and upper limits of the annual precipitation sum ($Paslimit.min,max$, mm; Bioclim12); the July precipitation sum ($P7slimit.min,max$, mm); the lower and upper limits of the annual mean monthly Thornthwaite agrometeorological aridity index ($TAIalimit.min,max$, $mm^{\circ}C^{-1}$); the lower and upper limits of the July Thornthwaite agrometeorological aridity index ($TAI7limit.min,max$, $mm^{\circ}C^{-1}$). Supplementary Table 1 shows the calibration of the climate models.

This means 7×2 bioclimatic factors in the model, in the case of both species, that is an acceptable number for distribution modelling purposes. For the numerical modelling of the potential distribution areas of species, these 7×2 extrema of climatic factors were used. Similarly, in the Pliocene-Pleistocene models, the modelling was based on the binary logic of the Boolean algebra that means that should indicate that the mathematical formalism of the deterministic unit step functions should also be similar:

$$\begin{aligned}
 1(Tam) &= \begin{cases} 0 & \text{if } Tam_{limit.min} > Tam > Tam_{limit.max} \\ 1 & \text{if } Tam_{limit.min} \leq Tam \leq Tam_{limit.max} \end{cases} \\
 1(T1m) &= \begin{cases} 0 & \text{if } T1m_{limit.min} > T1m > T1m_{limit.max} \\ 1 & \text{if } T1m_{limit.min} \leq T1m \leq T1m_{limit.max} \end{cases} \\
 1(T7m) &= \begin{cases} 0 & \text{if } T7m_{limit.min} > T7m > T7m_{limit.max} \\ 1 & \text{if } T7m_{limit.min} \leq T7m \leq T7m_{limit.max} \end{cases} \\
 1(Pas) &= \begin{cases} 0 & \text{if } Pas_{limit.min} > Pas > Pas_{limit.max} \\ 1 & \text{if } Pas_{limit.min} \leq Pas \leq Pas_{limit.max} \end{cases} \\
 1(P7s) &= \begin{cases} 0 & \text{if } P7s_{limit.min} > P7s > P7s_{limit.max} \\ 1 & \text{if } P7s_{limit.min} \leq P7s \leq P7s_{limit.max} \end{cases} \\
 1(TAIa) &= \begin{cases} 0 & \text{if } TAIa_{limit.min} > TAIa > TAIa_{limit.max} \\ 1 & \text{if } TAIa_{limit.min} \leq TAIa \leq TAIa_{limit.max} \end{cases} \\
 1(TAI7) &= \begin{cases} 0 & \text{if } TAI7_{limit.min} > TAI7 > TAI7_{limit.max} \\ 1 & \text{if } TAI7_{limit.min} \leq TAI7 \leq TAI7_{limit.max} \end{cases}
 \end{aligned}$$

where Tam represents the lower and upper limits of the annual mean temperature, $T1m$ is the georeferenced climate model data of the lower and upper limits of January mean temperature and $T7m$ is the georeferenced climate model data of the lower and upper limits of July mean temperature. Pas represents the georeferenced climate model data of the annual precipitation sum and $P7s$ is the georeferenced climate model data of the July precipitation sum. $TAIa$ is the annual mean monthly Thornthwaite agrometeorological aridity index, and $TAI7$ is the July Thornthwaite agrometeorological aridity index.

The potential areas can be determined according to the following mathematical formalism:

$$A(Tam; T1m; T7m; Tmc; Pas; Pswm; Psdm; Pscq)$$

$$= Tam \cap T1m \cap T7m \cap Pas \cap P7s \cap TAIa \cap TAI7$$

where $A(Tam; T1m; T7m; Pas; P7s; TAIa; TAI7)$ shows the potential distribution area of the given species, which contains the remaining areas after taking into consideration the temperature and precipitation limitations.

Identification of model environment B

This model environment was established to investigate the potential range of *Ph. sergenti* and *Ph. similis* during the Pannonian (Tortonian) era. The late Miocene Pannonian age model was based on the palaeoclimatic reconstruction of Bruch et al. (2006). The Pannonian is a Central Paratethys Stage that lasted from 11.6 to 6.15 Ma and equal to the Tortonian and lower Messinian era (see Table 1), but the analyses of Bruch et al. (2006) covered the ~11 to ~7 Ma section (=Pannonian sensu stricto) of these regional stratigraphic intervals. The palaeoclimatic reconstruction of Bruch et al. (2006) was based on 28 palaeofloras to obtain the following climatic variables: the annual mean temperature, the mean temperature of the summer months, the mean temperature of the winter months and the annual precipitation sum. The method of the palaeoclimatic reconstruction was based on the Coexistence Approach concept which was briefly presented in the identification of model A. The number of the involved sites was 17.

The used climatic variables were as follows: the lower and upper limits of the annual mean of the temperature (Tam ; °C; Bioclim1), the mean temperature of the warmest quarter ($Tmwa_{limit.min,max}$, mm; Bioclim10) and the mean temperature of the coldest quarter ($Tmc_{limit.min,max}$, mm; Bioclim11), as well as the lower and upper limits of the annual precipitation sum ($Pas_{limit.min,max}$, mm; Bioclim12) and the lower and upper limits of the annual mean monthly Thornthwaite agrometeorological aridity index ($TAIalimit.min,max$, $mm^{\circ}C^{-1}$); as can be seen, the limitation factor values are climate model-independent (Suppl.Tab. 2).

The difference value layers of the Pannonian (Tortonian) climatic variables from the present ones were created by inverse distance weighting (IDW) interpolation method which is mathematical assuming closer values are more related than further values with its function. Then, the reference period's climatic variable layers were modified by the identical interpolated difference value layers. To increase the number of the variables, the annual monthly average of the Thornthwaite agrometeorological aridity index was also calculated. Figure 2 a–d show the IDW interpolated maps of the primary four climatic variables.

This means 5×2 bioclimatic factors in the model in the case of both species that is an acceptable number for distribution modelling purposes. For the numerical modelling of the potential distribution areas of species, these 5×2 extrema of climatic factors were used. Similarly, in the above-described A- and B-type models, the modelling was based on the binary logic of the Boolean algebra:

$$\begin{aligned}
 1(Tam) &= \begin{cases} 0 & \text{if } Tam_{limit.min} > Tam > Tam_{limit.max} \\ 1 & \text{if } Tam_{limit.min} \leq Tam \leq Tam_{limit.max} \end{cases} \\
 1(Tmwa) &= \begin{cases} 0 & \text{if } Tmwa_{limit.min} > Tmwa > Tmwa_{limit.max} \\ 1 & \text{if } Tmwa_{limit.min} \leq Tmwa \leq Tmwa_{limit.max} \end{cases} \\
 1(Tmc) &= \begin{cases} 0 & \text{if } Tmc_{limit.min} > Tmc > Tmc_{limit.max} \\ 1 & \text{if } Tmc_{limit.min} \leq Tmc \leq Tmc_{limit.max} \end{cases} \\
 1(Pas) &= \begin{cases} 0 & \text{if } Pas_{limit.min} > Pas > Pas_{limit.max} \\ 1 & \text{if } Pas_{limit.min} \leq Pas \leq Pas_{limit.max} \end{cases} \\
 1(TAIA) &= \begin{cases} 0 & \text{if } TAIA_{limit.min} > TAIA > TAIA_{limit.max} \\ 1 & \text{if } TAIA_{limit.min} \leq TAIA \leq TAIA_{limit.max} \end{cases}
 \end{aligned}$$

where *Tam* represents the lower and upper limits of the annual mean temperature, the *Tmwa* is the georeferenced climate model data of the lower and upper limits of the mean temperature of the warmest quarter and *Tmc* is the georeferenced climate model data of the lower and upper limits of the mean temperature of the coldest quarter. *Pas* represents the georeferenced climate model data of the annual precipitation sum. *TAIA* is the annual mean monthly Thornthwaite agrometeorological aridity index.

The potential areas can be determined according to the following mathematical formalism:

$$\begin{aligned}
 A(Tam; Tmwa; Tmc; Pas; TAIA) \\
 = Tam \cap Tmwa \cap Tmc \cap Pas \cap TAIA
 \end{aligned}$$

where $A(Tam; Tmwa; Tmc; Pas; TAIA)$ shows the potential distribution area of the given species, which contains the remaining areas after taking into consideration the temperature and precipitation limitations.

Summarising the used climatic factors, a total of 14 variables was involved, but it varied by the model environments in which variables were used in the modelling in a given geological period (Table 2).

Identification of model environment C

The third model environment (*model environment C*) was performed to model the Pliocene and middle Pleistocene distribution of *Ph. sergenti* and *Ph. similis*. Pliocene and Middle Pleistocene models were run for both *Ph. sergenti* and *Ph. similis*. The steps of the modelling were as follows: the

Fig. 2 The IDW interpolated differences of the Pannonian regional stratigraphic unit-age climatic variables from the reference period's values ($\Delta value_{past\ climate\ model}$; $value_{1970-2000}$). **a** mean annual

temperature, **b** mean temperature of the summer months, **c** mean temperature of the winter months and **d** the annual precipitation sum

suitable climatic parameters were selected from the climate extrema results of Trájer et al. (2013) for modelling. Since the original climatic range limiting values data were given in monthly values, data was converted into bioclimatic variables according to the definitions of the WordClim database (Wordclim-Bioclimatic variables, n.d.). The used climatic variables were as follows: the lower and upper limits of the mean temperature of the wettest quarter ($Tmwe_{limit.min,max}$, °C; Bioclim8), the mean temperature of the driest quarter ($Tmd_{limit.min,max}$, °C; Bioclim9), the mean temperature of the warmest quarter ($Tmwa_{limit.min,max}$, mm; Bioclim10) and the mean temperature of the coldest quarter ($Tmc_{limit.min,max}$, mm; Bioclim11), as well as the lower and upper limits of the annual precipitation sum ($Pas_{limit.min,max}$, mm; Bioclim12), the precipitation sum of the wettest month ($Pswm_{limit.min,max}$, mm; Bioclim13), the precipitation sum of the driest month

($Psdm_{limit.min}$, mm; Bioclim14) and the precipitation sum of the coldest quarter ($Pscq_{limit.min}$, mm; Bioclim19). As can be seen, the limitation factor values are climate model-independent.

This means 10×2 bioclimatic factors in the model in the case of both species. For the numerical modelling of the potential distribution areas of species, the above-described extrema of climatic factors were used. There is a distribution function within the extrema which shows the distribution maximum for the given factor, but in the proposed approach the distribution of the internal interval is neglected, and the appearance of the species is characterised by true or false (0; 1) values according to the Boolean algebra.

Using climatic factors, deterministic unit step functions can be written in the following form:

Table 2 Summarisation of the used climatic variables in the case of the different model environments and species (* in the case of *Phlebotomus sergenti*, models were not generated in model environment A)

Variable	Denomination of the variable	Model A*	Model B	Model C
<i>Ph. sergenti</i>				
Tam	Annual mean of the temperature	—	—	x
T1m	January mean temperature	—	—	—
T7m	July mean temperature	—	—	—
Tmwe	Mean temperature of the wettest quarter	—	x	—
Tmd	Mean temperature of the driest quarter	—	x	—
Tmwa	Precipitation sum of the warmest quarter	—	x	x
Tmc	Mean temperature of the coldest quarter	—	x	x
Pas	Annual precipitation sum	—	x	x
P7s	July precipitation sum	—	—	—
Pswm	Precipitation sum of the wettest month	—	x	—
Psdm	Precipitation sum of the driest month	—	x	—
Pscq	Precipitation sum of the coldest quarter	—	x	—
TAIa	Annual mean monthly TAI	—	—	x
TAI7	July TAI	—	—	—
<i>Ph. similis</i>				
Tam	Annual mean of the temperature	x	—	x
T1m	January mean temperature	x	—	—
T7m	July mean temperature	x	—	—
Tmwe	Mean temperature of the wettest quarter	—	x	—
Tmd	Mean temperature of the driest quarter	—	x	—
Tmwa	Precipitation sum of the warmest quarter	—	x	x
Tmc	Mean temperature of the coldest quarter	—	x	x
Pas	Annual precipitation sum	x	x	x
P7s	July precipitation sum	x	—	—
Pswm	Precipitation sum of the wettest month	—	x	—
Psdm	Precipitation sum of the driest month	—	x	—
Pscq	Precipitation sum of the coldest quarter	—	x	—
TAIa	Annual mean monthly TAI	x	—	x
TAI7	July TAI	x	—	—

$$\begin{aligned}
1(Tmwe) &= \begin{cases} 0 & \text{if } Tmwe_{limit.min} > Tmwe > Tmwe_{limit.max} \\ 1 & \text{if } Tmwe_{limit.min} \leq Tmwe \leq Tmwe_{limit.max} \end{cases} \\
1(Tmd) &= \begin{cases} 0 & \text{if } Tmd_{limit.min} > Tmd > Tmd_{limit.max} \\ 1 & \text{if } Tmd_{limit.min} \leq Tmd \leq Tmd_{limit.max} \end{cases} \\
1(Tmwa) &= \begin{cases} 0 & \text{if } Twa_{limit.min} > Twa > Twa_{limit.max} \\ 1 & \text{if } Twa_{limit.min} \leq Twa \leq Twa_{limit.max} \end{cases} \\
1(Tmc) &= \begin{cases} 0 & \text{if } Tmc_{limit.min} > Tmc > Tmc_{limit.max} \\ 1 & \text{if } Tmc_{limit.min} \leq Tmc \leq Tmc_{limit.max} \end{cases} \\
1(Pas) &= \begin{cases} 0 & \text{if } Pas_{limit.min} > Pas > Pas_{limit.max} \\ 1 & \text{if } Pas_{limit.min} \leq Pas \leq Pas_{limit.max} \end{cases} \\
1(Pswm) &= \begin{cases} 0 & \text{if } Pswm_{limit.min} > Pswm > Pswm_{limit.max} \\ 1 & \text{if } Pswm_{limit.min} \leq Pswm \leq Pswm_{limit.max} \end{cases} \\
1(Psdm) &= \begin{cases} 0 & \text{if } Psdm_{limit.min} > Psdm > Psdm_{limit.max} \\ 1 & \text{if } Psdm_{limit.min} \leq Psdm \leq Psdm_{limit.max} \end{cases} \\
1(Pscq) &= \begin{cases} 0 & \text{if } Pscq_{limit.min} > Pscq > Pscq_{limit.max} \\ 1 & \text{if } Pscq_{limit.min} \leq Pscq \leq Pscq_{limit.max} \end{cases}
\end{aligned}$$

where *Tmwe* represents the georeferenced climate model data of the mean temperature of the wettest quarter, the *Tmd* is the georeferenced climate model data of the mean temperature of the driest quarter, *Tmwa* is the georeferenced climate model data of the precipitation sum of the warmest quarter and *Tmc* shows the georeferenced climate model data of the mean temperature of the coldest quarter. *Pas* represents the georeferenced climate model data of the annual precipitation sum, *Pswm* is the georeferenced climate model data of the precipitation sum of the wettest month, *Psdm* is the precipitation sum of the driest month and *Pscq* is the precipitation sum of the coldest quarter.

The temperature and precipitation climate models are available in a monthly resolution. The areas excluded by the limiting factors must be summed, and the intersection of the potential area designated by the factors gives the aggregated distribution area, formally:

$$\begin{aligned}
A(Tmwe; Tmd; Tmwa; Tmc; Pas; Pswm; Psdm; Pscq) \\
= Tmwe \cap Tmd \cap Tmwa \cap Tmc \cap Pas \cap Pswm \cap Psdm \cap Pscq
\end{aligned}$$

where $A(Tmwe; Tmd; Tmwa; Tmc; Pas; Pswm; Psdm; Pscq)$ shows the potential distribution area of the given species, which contains the remaining areas after taking into consideration the temperature and precipitation limitations.

Modelling results were displayed using Quantum GIS 3.4.4 software. The Lambert Azimuthal Equal Area (EPSG:3035) was used as a projection system in each case. The lower colour code limit of model A is 50%, and the lower limit of model B is 62%. The upper limit is 100% in each cases.

Model verification

Utilising the A and C model environments for the reference period's climate values, both *Ph. sergenti* and *Ph. similis*

presently can be found in those regions where the modelled suitability values exceed the 87–87.5% values. The modelled current distribution patterns of *Ph. sergenti* and *Ph. similis* do correspond well to the presently observed distributions of the two species (ECDC 2020). In the case of *model environment B*, it can be stated that the model environment is more permissive compared to the previous ones, and the maximum suitability value covers both the sporadic and permanent present occurrence of the species (Suppl.Fig.1). Based on these facts, *model environments A* and *C* can be handled as the permanent distribution models of the occurrence of the sandfly species, *model environment B* shows sensitively the borders of the sporadic occurrence, but this model is not able to make a difference between the permanent and the sporadic distribution areas.

Sampling grids

To quantify the mapped model results, the suitability values of the modelled sandfly species were sampled according to two grids. The first one was a so-called North Pontic grid' that covers the former northwest part of the 'East Paratethys grid (Fig. 3(a)). This grid was utilised in the Miocene suitability models of *Ph. similis*. The so-called East Paratethys grid covers the large areas of former East Paratethys (Fig. 3(b)).

Results

Phlebotomus similis in the North Pontic region (23.03–5.33 Ma)

In the Aquitanian and the Burdigalian (early Miocene) periods, the climatic suitability of the North Pontic area for the presently existing *Ph. similis* generally could be 68–74% (Fig. 4(a, b)). For the Tarkhanian (early-middle Langhian) period, this value could be between 62 and 74% (Fig. 4(c)). In the Badenian (Langhian and early Serravallian) models, the suitability values generally could range between 68 and 74% again (Fig. 4(d–f)). In the Sarmatian (late Serravallian) and the Maeotian 1 and 2 (late Tortonian–early Messinian), the modelled suitability values reach the 87% value in the southern shorelines (Fig. 4(g–i)), but in the Pontian (late Messinian) model, the maximums decrease to 74% (not including any small regions; Fig. 4(j)). It can be stated that between the Aquitanian and the Pontian (late Messinian) period, concerning the climatic requirements of the present *Ph. similis* populations, the climatic suitability of the North Pontic area never could reach the 100% value and only in the late Miocene area approximated the 90% value. Based on the verification models, the modelled maximally suitable areas are equal to the permanently inhabited regions by *Ph. similis*.

Fig. 3 The used sampling grids in the study. **a** ‘North Pontic grid’, **b** ‘East Paratethys grid’

In the Aquitanian, the three quarters of the sampled area could belong to the 74–80%, about the remaining part to the 81–86% suitability value intervals. In the Burdigalian and the entire Badenian (Langhian and early Serravallian), about 90% of the area could belong to the 74–80% suitability interval. In the Tarkhanian (early-middle Langhian), the most frequent suitability value in the investigated area could be 68–73%. In the Sarmatian (late Serravallian) and the Maeotian 1 (late Tortonian), about 10% of the areas could reach 87–93% suitability values. In the Maeotian 2 (early Messinian), 54% of the area could belong to the 81–93% interval. For the Pontian (late Messinian) period, this value could fall to 16%. It can be stated that the Sarmatian-Maeotian (late Serravallian-early Messinian) interval could provide the most suitable climatic conditions for *Ph. similis* in the North Paratethys area. Figure 5 shows the histographic depictions of the modelled suitability values.

Possible Pannonian (Tortonian) distribution areas

In the Pannonian (Tortonian), a major part of Asia Minor, the eastern parts of the Balkan Peninsula and the northwest part of the East Paratethys shoreline could be suitable for *Ph. sergenti* (Fig. 6(a)). In the case of *Ph. similis*, the modelled area of highly suitable regions is somewhat smaller, but the patterns are similar. For *Ph. similis*, the model displayed lower suitability values for the West Caspian region. Based on the verification models, the modelled maximally suitable areas are equal to the permanently and sporadically inhabited regions by the two species (Fig. 6(b)).

In the Pannonian (Tortonian), about 21 and 9% of the ‘North Pontic grid’ area could reach the maximum suitability value in the case of *Ph. sergenti* and *Ph. similis*. The 90–99% suitability values were the most frequent in both cases (Fig. 7).

Possible ranges in the mid-Pliocene and mid-Pleistocene periods

During the M2 mid-Pliocene glaciation (3.3 Ma), *Ph. similis* and *Ph. sergenti* could have very similar suitability patterns. Neither north nor south to the Ponto-Caspian area, the modelled suitability values do not show notable differences comparing the two species. Theoretically, at that time, both sandflies could inhabit the Atlantic and the southwest Baltic area and several parts of the Mediterranean Basin. The predicted suitability values are lower in the M2 mid-Pliocene glaciation era in those regions where the two species exist than in the western and east-central European regions. The Alps and the Carpathian Ranges could form notable geographic barriers against the migration of the species (Fig. 8(a, b)). In the mid-Pliocene warm period (3.264–3.025 Ma), the high

Fig. 4 The modelled suitability values of *Phlebotomus similis* in the North pontic area from the Aquitanian to the Pontian (upper Messinian) era **a** Aquitanian, **b** Burdigalian, **c** Tarkhanian (early-middle Langhian), **d** early Badenian (Langhian), **e** middle Badenian 2 (late Langhian), **f** late Badenian (early Serravallian), **g** Sarmatian (late Serravallian), **h** Maeotian 1 (late Tortonian), **i** Maeotian 2 (early Messinian), **j** Pontian (late Messinian)

suitability values of *Ph. similis* were restricted south to the Ponto-Caspian area in the Paratethys realm. *Ph. sergenti* shows somewhat higher suitability values also north to the Black Sea and in other areas of Europe, but the suitability profile of the two species could be similar. Both species show higher suitability values in the Pannonian Basin, but the suitability values of *Phlebotomus sergenti* could be higher. East to the former Caspian Sea, both species show very low suitability values. Theoretically, both species could occur in wide regions of Europe. The modelled suitability values are generally low in North Africa (Fig. 8(c, d)). In the MIS19 mid-Pleistocene (787 ka), high suitability values of *Ph. similis* could be restricted to the Balkans and the Northern regions of the Middle East in Southeast Europe. The modelled suitability patterns of *Ph. sergenti* could be very similar to *Ph. similis* that time. At the south distribution border, the suitability profile could be almost the same. That time, the Dinarids and the Alps could form notable barriers against the migration of the species between the Apennine and the Balkan Peninsulas (Fig. 8(e, f)). Based on the verification models, the modelled maximally suitable areas are equal to the permanently inhabited regions by the two species.

In the case of the M2 middle-Pliocene glaciation period, only a few regions could be suitable for both species on the north coasts of the East Paratethys area. In the mid-Pliocene warm period, a somewhat higher percentage area belongs to the highest suitability group, but the difference between *Ph. similis* and *Ph. sergenti* in the absolute number of the sampled values is moderate. In the selected Pleistocene period, 787 ka, neither *Ph. sergenti* nor *Ph. similis* could persist in the northern regions of the East Paratethys realm according to the model. The values also depend on the changing sea level patterns (Fig. 9).

Comparing the modelled suitability values of the different periods (pairs: Fig. 10(a, b): the M2 mid-Pliocene glaciation and the mid-Pliocene warm period; Fig. 10(c, d): the mid-Pliocene warm period and the MIS19 mid-Pleistocene period in 787 ka), it can be stated that the trends are similar whether we compare individual periods or species. It indicates that the climate of the Northeast Paratethys realm could be somewhat more suitable for *Ph. sergenti* than *Ph. similis* in general, but neither *Ph. sergenti* nor *Ph. similis* could be associated with this region. The differences in the modelled suitability values between the periods are quite similar comparing the two sandfly species in the modelled south distribution border.

Discussion

This is the first case in the literature when an originally phylogenetic-based Phlebotomine sandfly speciation theory was tested by multiple palaeobiogeographic models. The inter- and post-glacial migration and glacial refugia hypotheses of the Mediterranean sandfly were tested using a similar

approach using existing palaeoclimate models (Trájer and Sebestyén, 2019). However, this is also the first case when multiple regional palaeoclimate models were applied to investigate a speciation theory. This novelty in the speciation theory development of arthropod vector species is very important because due to the lack of fossil remains of evidence, the reconstruction of the speciation history of these arthropods requires a complex, phylogenetic-ecological, niche-based modelling approach.

A basic hypothesis of the adaptive speciation theories is that species should adapt to the permanently changing environmental conditions of the area where they occur; otherwise, the species would be extinct (Weissing et al. 2011). However, it is known that the environment permanently changes, that is the field of adaptive dynamic theories (e.g. Doebeli and Dieckmann 2005) and contra-theories (e.g. Waxman and Gavrillets 2005). The crucial problem of past ecological niche modelling is whether the present species have a similar niche to their ancestors or not. Several authors concluded in the twentieth century that theoretically, the selection-driven speciation will only occur under specific and extreme conditions and adaptive speciation should be relatively rare (Felsenstein 1981). In contrast, the more novel ecological speciation models suggest that selection-driven speciation is much more frequent under complex and permanently changing environmental conditions in nature than it was projected by former mathematical models (Weissing et al. 2011). The phylogenetic relations and the climatic requirements of certain Mediterranean taxa confirm the flexible adaptation capability of sandfly species to the changing climatic conditions. For example, *Phlebotomus sergenti*, *Ph. similis* and the non-*Paraphlebotomus* species *Phlebotomus* (*Phlebotomus*) *papatasi* Scopoli, 1786 belong to the same ‘East Mediterranean’ ecological group of Mediterranean sandflies. Because *Ph. similis* and *Ph. sergenti* are close relatives of each other within the subgenus *Paraphlebotomus* (Depaquit et al. 2002), as it was mentioned above, it is not plausible that a co-evolution process could explain the similarities in the climatic requirements of the species. However, it is questionable whether the above-mentioned three species became similar in their environmental demands by adapting to similar conditions, or it is an ancient, plesiomorph ecological character of Phlebotomine sandflies.

A possible, adequate solution to the problem would be that the distribution limiting climatic demands of *Ph. similis* notably changed in the last million years. However, this presumption is contradicted by the fact that the climatic requirements of the sister species *Ph. sergenti* is very similar to the demands of *Ph. similis* (Trájer et al. 2013). If *Ph. similis* should adapt to the subtropical humid environment of the Miocene North Paratethys, the extant

Fig. 5 The histogram of the relative frequencies of the modelled suitability values of *Phlebotomus sergenti* and *Phlebotomus similis* in the ‘North Pontic grid’ in the Miocene epoch. The colour codes are the same as those used in Fig. 4

species could have merely different climatic requirements compared to *Ph. sergenti*. If the ancestor of *Ph. similis* inhabited the northern regions of the Paratethys; it would be expected that this species would be more tolerant of a cooler environment and

higher annual precipitation than *Ph. sergenti*. In present-day Europe, *Phlebotomus* (Larroussius) *ariasii* Tonnoir, 1921 and *Phlebotomus perniciosus* Newstead, 1911 have such kind of

Fig. 6 The modelled suitability of *Phlebotomus sergenti* (a) and *Phlebotomus similis* (b) in the Pannonian (Tortonian; 10 Ma) in the East Mediterranean Basin and East and Central Paratethys realm

Fig. 7 The histogram of the relative frequencies of the modelled suitability values of *Phlebotomus sergenti* (a) and *Phlebotomus similis* (b) in the ‘North Pontic grid’ in the Pannonian (Tortonian) era. The colour codes are the same as those used in Fig. 6

climatic tolerance characters (Trájer et al. 2013), but in the case of *Ph. similis*, it is not the fact.

If the adaptation flexibility of Phlebotomine species could be high, it would imply that sandfly species could be able to adapt quickly to the changing environments. This assumption is not supported by the essentially warm climate-dependent worldwide distribution of the sandfly species. In certain cases, sandflies could adapt quickly to new environments. Rosário et al. (2017) documented that several New World sandfly species were adapted to the urbanised regions of Brazil in the last few decades. However, it should be noted that warmer, nutrient-rich and predator-poor habitats provided by cities are favoured by sandfly species. It seems that the glacial-interglacial cycles of the last few million years may not notably affect the ecological limits of sandflies. Several authors showed that the range of the Mediterranean sandflies during the glacials of the last half-million year was restricted to South European and Asian Minor refugees (e.g. Mahamdallie et al. 2011; Akhoundi et al. 2016). It is also notable that those sandfly species which still occur in the warmer continental areas of Europe only survive in special, protected environments like in the protected walls of abandoned quarries (Trájer et al. 2018b). On the other hand, the overwintering and development of the Mediterranean sandfly larvae require high temperatures which rather evoke conditions typical of Miocene-Pliocene periods in Europe. For example, the lower temperature limit of *Ph. papatasi* larvae in Greece is about -4°C , and the optimum is about $28\text{--}34^{\circ}\text{C}$; the lower temperature limit of *Ph. sergenti* larvae are unknown, but the optimum is about $31\text{--}33^{\circ}\text{C}$. These survival and development-limiting values are characteristic of subtropical arthropods, not for continental taxa (Lindgren et al. 2006). It is worth recalling that the hypothesis that the present species share several common characters in their ecological needs with their Neogene ancestors is an accepted idea. For example, the Coexistence Approach itself is also based on the principle of actualism (Mosbrugger and Utescher

1997). It is based on the central hypothesis that the distribution limiting requirements of the flora elements did not change markedly (in most of the species) during the Neogene. Trájer et al. (2018A) showed that *Ph. similis* and *Ph. sergenti* was adapted to the hot, dry summer Mediterranean climate during the last million years. In any case, all the 16 Neogene age fossil taxa of the New World Phlebotominae (*Lutzomyia*, *Micropygomyia*, *Pintomyia* and *Psathyromyia*) species were found in those regions (Dominica and Mexico; Poinar 2008; Ibañez-Bernal et al. 2014), where the climate was tropical-subtropical during the Neogene era. Because these genera are close relatives of the Old World Phlebotomine sandflies, it could be hypothesised that Phlebotomine sandflies originally were adapted strictly to the tropical-subtropical environments which were also proposed by Ready (2000) and this trait of the species has not changed significantly during the Neogene era.

The modelled former ranges of the studied two *Paraphlebotomus* species call into question the formerly described hypothesis that the speciation of *Ph. sergenti* and *Ph. similis* could be the consequence of the geographical segregation of their former *Paraphlebotomus* populations around the Central and East Paratethys realm (Depaquit et al. 1998, 2000, 2002; Essegir et al. 2000). The modelled suitability values of *Ph. similis* never reached the maximum suitability value in the Miocene epoch in the northwest part of the East Mediterranean Basin in the *model environment A*. In fact, the values never overwhelmed the 93% value. It is curious if we hypothesise that *Ph. similis* were adapted to the climatic conditions of the North Paratethys and the Southward migration of the species to its present distribution area originated from this region. From the Aquitanian to the Badenian (Langhian and early Serravallian) period, the climate of the area was too humid for *Ph. similis* along the north shoreline of the Paratethys (compare the limiting and the climatic values presented in Table 3 and Supplementary Table 1) and it is very plausible that the ancestor of *Ph. similis* could not be able to colonise the Northwest shoreline of the Paratethys. This presumption is also supported by the

Fig. 8 The modelled suitability of *Phlebotomus similis* (a) and *Phlebotomus sergenti* (b) in the M2 mid-Pliocene cold period (3.3 Ma) in western Eurasia and the East and Central Paratethys realm. The modelled suitability of *Phlebotomus similis* (c) and *Phlebotomus sergenti* (d) in the mid-Pliocene warm period (3.264–3.025 Ma) in western

Eurasia and the East and Central Paratethys realm. The modelled suitability of *Phlebotomus similis* (e) and *Phlebotomus sergenti* (f) in the MIS19 mid-Pleistocene period (787 ka) in western Eurasia and the East and Central Paratethys realm

former Miocene flora elements. It is known that there is a connection between the environmental requirements of the extant Mediterranean sandfly taxa and flora elements (Bede-Fazekas and Trájer 2013). For example, in Central Paratethys, the Pannonian Domain had during the Badenian (Langhian and early Serravallian) a typical warm and humid climate-dwelling flora that became a warm temperate one in the late Miocene era (Erdei et al. 2007). Similar climatic and floral trends were observed by Jiménez-Moreno (2006) during the middle Miocene in Central Europe, and by Ivanov et al. (2011) in the East Paratethys Basin in the Miocene. The fossil late Miocene (Pannonian=Tortonian) age flora of Pécs-Danitzpuszta, Hungary

suggests thermophilous, Mediterranean laurophyllous (evergreen broadleaf) vegetation with evergreen *Quercus* species (Hably and Sebe 2016) that could provide very similar conditions where the extant Mediterranean sandfly species live (Trájer et al. 2013). It also indicates that the late Miocene could be the first time when the *Paraphlebotomus* species could inhabit the shorelines of the Central and East Paratethys area.

It is also not plausible that *Ph. similis* became similar to *Ph. sergenti* after the disintegration of the Paratethys Sea, because the common distribution area of the two species is relatively small, which is not notable if we see the total range of *Ph.*

Fig. 9 The histogram of the relative frequencies of the modelled suitability values of *Phlebotomus sergenti* and *Phlebotomus similis* in the ‘East Paratethys grid’ in the M2 mid-Pliocene cold and warm periods

and in the MIS19 mid-Pleistocene period. The colour codes are the same as those used in Fig. 8

similis. Nevertheless, the climatic requirements of *Ph. sergenti* is very similar to that *Ph. similis* has (Trájer et al. 2013). Using the Occam’s razor principle that postulates that conceptual entities should not be multiplied without necessity (Blumer et al. 1990), it is much more plausible that both *Ph. similis* and *Ph. sergenti* have largely preserved the environmental needs of their common Asian ancestors that were adapted

plausibly to summer dry and hot subtropical climates (Trájer et al. 2018a). If, however, the earlier speciation theory is true, then the climate of the Sarmatian (late Serravallian) and the Maeotian (late Tortonian-early Messinian) was the most suitable for *Ph. similis*, but in this case, it is worth emphasising that the modelled suitability values are lower than 94% and restricted to a relatively narrow coastline area. It is

Fig. 10 Changes of the modelled suitability values between the M2 mid-Pliocene cold and warm periods ($\Delta\text{value}_{\text{mid-Pliocene warm period model; value-mid-Pliocene M2 cold period model}}$; **a** *Phlebotomus similis*, **b** *Phlebotomus sergenti*); differences in the modelled suitability values between the

mid-Pliocene warm and the MIS19 mid-Pleistocene (787 ka) periods ($\Delta\text{value}_{\text{mid-Pliocene warm period model; value-mid-Pleistocene MIS19 period model}}$; **c** *Phlebotomus similis*, **d** *Phlebotomus sergenti*)

questionable whether the species could have been present at all in the North Paratethys region. According to the current models, in areas where the suitability value just reaches 87%, *Ph. similis* and *Ph. sergenti* only sporadically can occur. For

example, *Ph. similis* occur under the humid subtropical climate of the coastal regions of the Crimean Peninsula, forming only 0.1% of the sandfly fauna (Baranets et al. 2019). It raises the possibility that *Ph. similis* is only an introduced species in

Table 3 The used bioclimatic distribution limiting values (T in °C, P in mm, TAI in mm/°C)

Variable	Denomination of the variable	Min (<i>Ph. sergenti</i>)	Max (<i>Ph. sergenti</i>)	Min (<i>Ph. similis</i>)	Max (<i>Ph. similis</i>)
Tam _{limits}	Annual mean of the temperature	9.0	18.9	9.4	18.0
T1m _{limits}	January mean temperature	-0.7	10.8	-0.1	20.2
T7m _{limits}	July mean temperature	19.2	28.8	10.0	28.3
Tmwe _{limits}	Mean temperature of the wettest quarter	5.1	15.7	5.4	15.4
Tmd _{limits}	Mean temperature of the driest quarter	18.1	28.0	18.7	27.2
Tmwa _{limits}	Precipitation sum of the warmest quarter	18.1	28.0	18.7	27.2
Tmc _{limits}	Mean temperature of the coldest quarter	1.1	11.8	1.4	10.5
Pas _{limits}	Annual precipitation sum	105	1140	144	1038
P7s _{limits}	July precipitation sum	0	27	0	27
Pswm _{limits}	Precipitation sum of the wettest month	36	363	27	180
Psdm _{limits}	Precipitation sum of the driest month	0	96	0	24
Pscq _{limits}	Precipitation sum of the coldest quarter	51	450	72	414
TAIa _{limits}	Annual mean monthly TAI	0.62	5.71	0.86	5.31
TAI7 _{limits}	July TAI	0	1.27	0	1.35

Crimea or this area belongs to the unstable, sporadic range of the species.

It was found that *Ph. similis* does not have a better adaptation to the cooler and wetter climatic conditions of the mid-Pliocene and mid-Pleistocene interstadial North Pontic area than of *Ph. sergenti*, which also manifests in the very similar northern modelled and the observed permanent distribution areas in the Southeast Mediterranean area. The southern distribution borders are also quite similar, which is a curious circumstance if one of the species was originally adapted to the somewhat cooler climate of the north Paratethys coastal areas. When the contiguous marine body of the Paratethys still existed, the distance between the north and the south coasts of the Paratethys could overwhelm the 800 km in the eastern basin in the north to south direction. Such a great longitudinal distance theoretically could result in notable climatic differences between the two sides of the Paratethys. Bruch et al. (2007) showed that in the early late and middle Miocene era, the annual mean temperature of Asia Minor was about 17–19 °C, the same climatic value in the North Paratethys area could be ‘only’ 14–16 °C. It shades the image that the north part of the Middle East could have elevated, mountainous topography, even in the late Miocene and the Pliocene. This geographical condition causes the alternating pattern of cool highland and relatively warm valley areas in present-day Turkey and Iran and the territory of the Caucasian states (in this region, the annual mean temperature could be 16–17 °C). However, this difference cannot explain the very similar climatic needs of the two species if *Ph. similis* evolved along the north coasts of the former Paratethys.

The outcomes of the *model environment B* indicated that both *Ph. sergenti* and *Ph. similis* could occur in the northwest shoreline of the East Paratethys Basin where theoretically *Ph. similis* could be evolved. Although due to the previously mentioned accuracy of this model environment, it can only be said that both species could occur sporadically or permanently in this region during the Tortonian. The results of the *model environment B* somewhat contradict the results of certain parts of the *model environment A* and *C* models. The reason for the difference can be found in the accuracy of the models. Even the models were based on similar methods and share some common factors; it cannot be expected that the outcomes of the models be the same. It is an important difference between the models, that the number of the used climatic limits is different: it is 14 in the case of *model environment A*, 10 in *model environment B* and 16 in *model environment C*. As it could be expected, while the results of model environment B are relatively permissive and, in the case of the present period, covers also the sporadic ranges of the species, *model environment A* and *C* are more stringent and accurately show both the permanently and the sporadically inhabited regions of the two species.

The model of Koch et al. (2017), which was based on the occurrence data of Artemiev and Neronov (1984), also showed this wider distribution of *Ph. similis* which also contains the area of the sporadic observations due to the nature of the data source of sandfly distribution. This fact is very important in the evaluation of the outcomes of the models. Because the maximal suitability values of *model environment B* show the borders of the sporadic distribution, and these borderline areas can well correspond to the 87% value of *model environment A*, it can be stated that *Ph. similis* plausibly were not permanently present at the present-day North Pontic area during the late Miocene. It is also supported by the Maeotian 1 and 2 (late Tortonian–early Messinian) models of model environment B which shows the same patterns but were based on a different palaeoclimatic reconstruction. It is also very important that the *model environment B* was based on interpolated values and did not contain South Ukrainian palaeoclimatic data. Based on this fact, the outcomes of *model environment A* could be handled as the accurate model environment for the prediction of the Miocene distribution of *Ph. similis*, including the Tortonian era. Based on the above-described considerations, the continuously updated mapped Phlebotomine sandfly distribution maps of the European Centre for Disease Prevention and Control (ECDC 2020) provide more reliable information of the persistent occurrence of sandfly species than the data of Artemiev and Neronov (1984) and the European database is a more reliable base of ecological modelling. On the other hand, models confirm that the present Crimean populations of *Ph. similis* could not be the remnants of old, late Neogene populations because, in the Last Glacial Maximum (~21 ka), the area was too cold for the continuous persistence of the species (Trájer and Sebestyén 2019).

The Messinian Salinity Crisis–related speciation theory of the Mediterranean *Paraphlebotomus* species (Esseghir et al. 2000; Kasap et al. 2015) must also face some contradictions. First, it should be added that there is no doubt that the Messinian Crisis played a very important role in the divergence of the different lineages of the species. But it may be not true for the speciation of the *Paraphlebotomus* species. It seems that *Phlebotomus* (*Paraphlebotomus*) *kazeruni* Theodor and Mesghali, 1964, *Ph. jacusieli*, *Ph. similis* and *Ph. sergenti* still existed when the Messinian Salinity crisis led the sandfly populations to migrate from the continents to the islands and peninsulas. It can be hypothesised that because of *Ph. similis* could migrate to Malta, *Ph. sergenti* Cyprus, Sicily, and the Iberian Peninsula and the only period in the Neogene when these migrations could be possible was the Messinian Salinity Crisis. Maybe *Ph. similis* migrated to a land bridge of the sinking Hellenic Orogeny somewhat earlier (Fig. 11).

Fig. 11 The modified most parsimonious phylogram of *Phlebotomus kazeruni*, *Phlebotomus jacusieli*, *Phlebotomus similis* and *Phlebotomus sergenti* according to Depaquit et al. (2002). Empty star: continent-inland migration related to the presence of the land bridge of the Hellenic Orogenic Belt, filled star: continent-inland migration related to the Messinian desiccation of the Mediterranean Basin, purple highlight: the interval of the Messinian migrations in the phylogram. Note that the ends of the branches are not fixed

It is also a more problematic point of the previous theory that if *Ph. similis* originally adapted to the North Paratethys environment, this area could provide the highest suitability values in one of the periods of the Miocene epoch. In contrast, the modelled suitability values indicate that this area at most could belong to the northern distribution zone of the species in 9.0–5.3 Ma and not the speciation centre of the species. However, because the divergence of the two species should occur somewhere in the East Mediterranean area according to the phylogenetic studies (Esseghir et al. 2000), the explanation of the present, partly overlapping range and the species requires the explanation. Because the modelled south distribution borders of the two species are quite similar in each period, it can be hypothesised that (1) the speciation of the two species was very rapid, their geographical isolation was relatively episodic and the formerly hypothesised north Paratethyan species saved their adaptation to the drier and hotter Tethyan Mediterranean conditions; or (2) *Ph. similis*

evolved from the common ancestor in similar climate regions where the ancestor lived, but a geographical barrier created the patterns of the divergence of the populations of *Ph. sergenti* and *Ph. similis*. To localise the origin of *Ph. similis*, the present ranges and the climatic distribution limiting values of the two species provide the basis: (1) the climatic requirements of the two species are very similar and despite the similar niche, the two species do not displace each other in Southeast Europe and Asia Minor; (2) while *Ph. sergenti* has circum-Mediterranean range, *Ph. similis* is restricted to the Aegean area. According to these facts, it can be hypothesised that *Ph. similis* evolved close to its recent distribution area. Based on the model results and the phylogenetic evidence, the rethought steps of the speciation of the studied sandfly species were as follows:

- 1) In the early or middle Miocene Period, the ancestors of *Ph. jacusieli*, *Ph. sergenti* and *Ph. similis* invaded the East Tethys and South Paratethys area (Fig. 12(a)).
- 2) During the late Miocene Period, the tectonic subsidence of the Hellenic Orogenic Belt divided the sandfly populations of the Balkan and Asia Minor. The speciation of *Ph. jacusieli*, *Ph. sergenti* and *Ph. similis* happened after this event. One of the land bridges of the Hellenic Orogeny plausibly existed between Crete and the Peloponnese

Fig. 12 The steps of the migration and speciation of the ancestors of *Phlebotomus jacusieli*, *Phlebotomus sergenti* and *Phlebotomus similis* (a) early or middle Miocene Period, (b) late Miocene Period, (c)

uppermost stage of the Miocene (Messinian) Period, (d) a Pliocene cold period. *Phlebotomus kazeruni* was not depicted

- Peninsula when *Ph. similis* migrated to Crete some million years before the Messinian Crisis (Fig. 12(b)).
- 3) In the last Miocene period, in the Messinian period, the water table of the Tethys (the Mediterranean) Sea disappeared and the pathways opened for *Ph. similis* to migrate to Asia Minor and Malta. That time, *Ph. similis* migrated to Crete, the Balkan Peninsula and Cyprus and the Hispanian Peninsula through the Betic-Rif Orogenic Belt (in the location of the present-day Gibraltar Strait). Sporadically, *Ph. similis* could occur in the northwest part of the East Paratethys Basin (Fig. 12(c)).
 - 4) After the final flood of the Mediterranean Basin in the Zanclean, the continental and insular populations became isolated again from the other populations of the ancestor species as it was proposed by Kasap et al. (2015) (Fig. 12). *Paraphlebotomus* species became rare or extinct only after the mid-Pleistocene cooling in East Europe and the Pannonian Basin (Fig. 12(d)).

It is important to note that the outputs of this study do not contradict previous phylogenetic studies but provide an alternative interpretation of the speciation of *Ph. similis* and its sister taxa. It should be noted that Depaquit et al. (2000) originally did not investigate the phylogenetic relationships of the proposed Crimean, Romanian and Serbian *Ph. similis* populations because entomological materials from these areas were not involved in their study. Seeing the other results of their study, for example, the migration theory of *Ph. similis*, their results form the basis of understanding the present phylogenetic and distribution patterns of *Ph. sergenti*.

Conclusions

The model results do not provide hard evidence for the previously hypothesised North Paratethyan speciation of *Phlebotomus similis* in the Miocene or Pliocene epochs, but neither falsifies that. The northwest part of the former East Paratethys Basin could not be the speciation area of *Ph. similis*, because the only sporadic occurrence of the species can be hypothesised in the late Miocene era in this area. It was concluded that *Ph. sergenti* and *Ph. similis*, that sister *Paraphlebotomus* species are allopatric presently in the Balkan Peninsula and Asia Minor, should diverge from each other before the Messinian Salinity Crisis, perhaps at the turn of the middle and late Miocene era. However, further studies needed to test whether the hot and dry conditions of the former dry Mediterranean abyssal plain could allow the cross-basin migrations of sandfly taxa or not during the Messinian Period. Our model results indicate that the two species inhabited similar climate regions in the Miocene epoch and the Middle Miocene subsidence of the Hellene Orogenic Belt created the circumstances of the speciation of *Ph. similis*. This hypothesis can

explain why the distribution limiting climatic factors of *Ph. sergenti* and *Ph. similis* are so similar to each other. The resulting Aegean Sea divided the ancient *Paraphlebotomus* population into east, west and southwest sub-populations, as would have been the case if the Paratethys Sea could be the geographic barrier.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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