Bulletin of Insectology ***65** (1): 23-27, 2012*
ISSN 1721-8861

Study on siphonal measurements and usefulness in delimitation of "rural" and "urban" ecotypes of *Culex pipiens*

Bosiljka KRTINIĆ[1], Jasmina LUDOŠKI[2], Vesna MILANKOV[2]
[1]*Ciklonizacija, Novi Sad, Serbia*
[2]*Department of Biology and Ecology, Faculty of Sciences, University of Novi Sad, Serbia*

Abstract

Mosquitoes of the *Culex pipiens* complex (Diptera Culicidae) are of great medical and veterinary importance as vectors for various bacterial, filarial and viral diseases. Taxonomic status, biology and ecology of members of the *C. pipiens* complex are still matter of study and discussion and their morphological, physiological, genetic and ecological characteristics are imprecise and often controversial. The aim of this study was to compare the usefulness of different siphonal measurements in delimitation of "rural" and "urban" ecotypes of *C. pipiens*. In this study, three samples from different biotopes (underground - ecotype I; aboveground - ecotypes II and III) were analysed for morphological variation using nine siphonal indices - SI (as a ratio of the length to the width measurements) and one width index - WI (representing the ratio of the width of the base and top of the siphon). Highly significant differences among the three ecotypes in all siphonal indices (SI1-SI9) were observed. It was revealed significant differences (Tukey post hoc test) between ecotype III versus I and II. Within each ecotype CV values for all nine siphon indices were similar, with the exception of WI. Therefore, usefulness and sensitivity of nine SI studied are equal.

Key words: *Culex pipiens*, Culicidae, ecotype differentiation, siphonal index, siphonal variability.

Introduction

The members of the *Culex pipiens* complex are of medical and veterinary importance as vectors for various bacterial, filarial and viral diseases (Vinogradova and Shaikevich, 2007; Becker *et al*., 2010). Understanding the population structure and gene flow among populations of mosquito species is critical for public health issues such as local dispersion patterns, evolution and spread of insecticide resistance alleles, epidemiology of mosquito-borne pathogens and developing and testing management strategies and control of vector borne diseases (Tabachnick and Black, 1995; Smith *et al*., 2005; Rasgon *et al*., 2006).

The members of the *C. pipiens* complex represent one of the outstanding problems in the current mosquito taxonomy, with opinions on *Culex pipiens* L. taxonomy ranging from distinct species to physiological forms with considerable genetic introgression (Cornel *et al*., 2003). Taxonomic status, biology and ecology of members of the complex are still matter of study and discussion and their morphological, physiological, genetic and ecological characteristics are imprecise and often controversial (Harbach *et al*., 1985; Chevillon *et al*., 1995, 1998; Vinogradova, 2003; Fonseca *et al*., 2004; Kent *et al*., 2007; Gomes *et al*., 2009; Weitzel *et al*., 2009; Kothera *et al*., 2010). Members of *C. pipiens* occur in two forms: biotype *pipiens* ("rural") and biotype *molestus* ("urban"), separated based on physiological, behavioral characteristic and differences of structural genes. The form *pipiens* is anautogenous and eurygame, it has a reproductive diapause and feeds mostly on birds, even though the number of its anthropophilic populations has been increasing recently (Spielman, 1967, 2001; Harbach *et al*., 1985; Byrne and Nichols, 1999; Hamer *et al*., 2008). The form *molestus* is autogenous and stenogame, it cannot enter diapause and prefers to feed on humans (Spielman, 1967; Harbach *et al*., 1984; Byrne and Nichols, 1999; Vinogradova and Ivnitsky, 2009). The anautogenous biotype occurs mainly in aboveground and rural habitats, while the autogenous biotype occurs in the underground and also aboveground urban habitats (Ribeiro *et al*., 1983; Harbach *et al*., 1984; Gomes *et al*., 2009; Reusken *et al*., 2010). Besides on biological and behavioral diagnostic characters, molecular markers provide useful tools to identify *molestus*, *pipiens* and their hybrids. For example, based on fixed allelic differences and significant differences in the allele frequencies at the allozyme loci *Aat*, *Hbd*, *Mpi*, *Pgm*, *Ak*-1 and *Hk* (Chevillon *et al*., 1998; Becker *et al*., 1999; Weitzel *et al*., 2009) two *C. pipiens* forms were distinguished. In addition, one microsatellite locus was proposed as promising diagnostic marker (Bahnck and Fonseca, 2006; Kent *et al*., 2007; Gomes *et al*., 2009).

Morphological characters have long been in use in solving the taxonomic status of members of the *C. pipiens* complex. The respiratory siphon, a process of the eighth abdominal segment, is a valuable diagnostic character for identification and distinguishing genera and species within the family Culicidae (Becker *et al*., 2010). One of the oldest and commonly used taxonomic characters is the siphonal index - SI (the ratio of the length of the siphon to its width). Although the great variations in the values of this character have caused doubts about its diagnostic validity in the determination of members of the complex (McMillan, 1958; Harbach *et al*., 1984) this parameter, with certain modifications, continues to be used in the separation of species *C. pipiens* and *Culex quinquefasciatus* Say (Ishii, 1980; Brogdon, 1981, 1984; Kruppa, 1987; Cornel *et al*., 2003), as well as *C. pipiens* and *Culex torrentium* Martini (Service, 1968; Dahl, 1988; Vinogradova *et al*., 1996; Vinogradova and Ivnitsky, 2009) and biotypes *pipiens* and *molestus* (Vinogradova, 2003). However, consistent measures used for SI is lacking, which caused difficulties in comparison of published results. For example, measures in use for siphon width were: the middle of siphon (Belkin, 1962; Harbach and Knight, 1980) or widest part of the siphon at its base not counting acus (Gutsevich *et al*., 1974; Jupp, 1978; Cranston *et al*., 1987; Eritja and Aranda, 1995; Vinogradova *et al*., 1996). For siphon length, from base to tip excluding siphon valve, in use is: measurement along the middle of siphon (Brogdon, 1981; Cranston *et al*., 1987), along the posterior side (Gutsevich *et al*., 1974; Vinogradova *et al*., 1996) or not clearly defined (Eritja and Aranda, 1995; Cornel *et al*., 2003).

The study presented herewith is a part of a larger research project to quantify the spatial and temporal distribution of genetic and phenotypic variation of "urban" and "rural" ecotypes of *C. pipiens.* The current study was undertaken to test usefulness of different measures of length and width of the siphon as a standard measure of SI. Such different siphonal indices were used in delimitation of simpatrically occurring "rural" and "urban" ecotypes of *C. pipiens*.

Materials and methods

Larvae of *C. pipiens* were collected from a broader area of the city of Novi Sad (45°15'N; 19°50'E) (Autonomous Province of Vojvodina, northern part of Serbia) during September 2009, from three different biotopes: 1. street manhole (the underground habitat of urban type; ecotype I), 2. draining ditch and pond in the city (aboveground habitat of urban type; ecotype II) and 3. pond (swamp) outside urban area (aboveground habitat of rural type; ecotype III). Larvae of EIII were collected in Special Nature Reserve "Koviljsko-Petrovaradinski rit" with a complex of marshes and forest ecosystems (in 2004 it was included in the list of important water-related protected areas in the Danube basin - ICPDR, since 2005 this wetland is IPA - Important Plant area, and also a Ramsar Site candidate). Specimens of each biotype were collected at the same breeding site and time. Numerous adults and larvae were present on each locality at the time of sampling, which imply that sampled larvae had been obtained from many females. From each breeding site, around 300 larvae were collected and reared in the laboratory in their original water without feeding. After adult eclosion, exuviae of 4th instar larvae were preserved in 96% ethanol. Since exuviae (not larvae) were used for the study, all individuals were at the same point of development and that allowed using adults for sex determination and for further genetic and morphological analyses. In this study, a total of randomly chosen 144 exuviae (72 female and 72 male specimens) were analyzed. Due to lack of statistically significant differences between sexes found in a preliminary study of siphonal character variability, in further analysis genders were not analyzed separately. Specimens were identified based on the morphological characters of adults, defined for *C. pipiens* (Gutsevich *et al*., 1974; Božičić, 1985).

Each larval exuvia was placed in watch glass in a drop of water in the lateral position, and then photographed with a digital camera Leica DFC320 associated with a stereo microscope Leica MZ12.5, and the images were used in further morphometric analysis. Exuviae were not mounted in order to avoid being flattened.

Siphonal index (SI) variation was observed from 54, 48 and 42 specimens from ecotype I, II and III, respectively. Using Leica Application Suite Measurement Module (ver. 2.4.0) three length values (L1, L2, L3) and three width values (W1, W2, W3) on each siphon were measured (figure 1). Ten indices were calculated as a ratio of the measured linear distances - nine indices named siphonal indices (SI1-SI9) representing the ratio of the length to the width measurements (SI1 = L1/W1; SI2 = L1/W2; SI3 = L1/W3; SI4 = L2/W1; SI5 = L2/W2; SI6 = L2/W3; SI7 = L3/W1; SI8 = L3/W2; SI9 = L3/W3) and one index named width index (WI) representing the ratio of the width of the base and top of the siphon (WI = W1/W3) (figure 1).

Descriptive statistics (mean, standard deviation, minimal and maximal values, coefficient of variation) were calculated for each of 10 indices and differences among ecotypes were tested using one-way analysis of variance (ANOVA with the post hoc Tukey HSD test). All statistical analyses were done using Statistica for Windows (version 9.1).

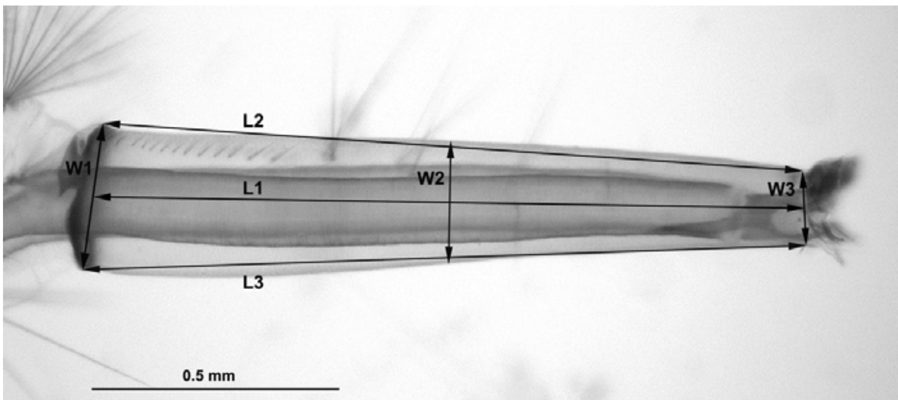

Figure 1. Length (L1, L2, L3) and width (W1, W2, W3) measurements of siphon of *C. pipiens*.

Table 1. Siphonal (SI1-SI9) and width (WI) indices for "urban" (I and II) and "rural" (III) ecotypes of *C. pipiens* from Novi Sad. For SI1-SI9 Tukey post hoc test revealed significant differences (p < 0.001) between ecotype pairs I/III and II/III.

Index	Ecotype I (n = 54)			Ecotype II (n = 48)			Ecotype III (n = 42)			ANOVA
	Mean (SD)	Min	Max	Mean (SD)	Min	Max	Mean (SD)	Min	Max	p
SI1	4.735 (0.457)	3.867	5.829	4.851 (0.493)	3.956	6.092	5.491 (0.443)	4.810	6.505	< 0.001
SI2	4.624 (0.454)	3.767	5.702	4.752 (0.490)	3.846	5.985	5.409 (0.451)	4.714	6.477	< 0.001
SI3	4.853 (0.457)	3.991	5.959	4.959 (0.496)	4.082	6.200	5.580 (0.435)	4.913	6.541	< 0.001
SI4	5.510 (0.531)	4.408	6.619	5.774 (0.679)	4.475	7.579	6.656 (0.648)	5.475	8.581	< 0.001
SI5	5.382 (0.525)	4.279	6.453	5.655 (0.670)	4.351	7.445	6.556 (0.649)	5.412	8.543	< 0.001
SI6	5.648 (0.534)	4.554	6.785	5.902 (0.688)	4.618	7.713	6.764 (0.646)	5.567	8.629	< 0.001
SI7	8.937 (0.723)	7.229	11.267	9.207 (1.071)	7.475	12.092	10.534 (0.767)	9.416	12.780	< 0.001
SI8	8.729 (0.716)	7.088	10.984	9.018 (1.053)	7.350	11.878	10.376 (0.774)	9.267	12.723	< 0.001
SI9	9.161 (0.728)	7.394	11.549	9.413 (1.090)	7.638	12.305	10.705 (0.764)	9.559	12.851	< 0.001
WI	1.893 (0.103)	1.656	2.206	1.898 (0.116)	1.614	2.125	1.922 (0.098)	1.671	2.088	0.386

Results and discussion

Descriptive statistics for ten analyzed indices were given in table 1. For each siphonal index (SI1-SI9) it was shown that ecotype III specimens had the highest mean index value comparing to specimens of ecotypes I and II (table 1). The ANOVA showed highly significant differences (p < 0.001) among the three ecotypes in all siphonal indices (SI1-SI9). Tukey post hoc test revealed significant differences (p < 0.001) between ecotype III versus I and II. Contrary to siphonal indices, width index (WI) among ecotypes revealed no significant differences ($F_{(2,14)} = 0.96$; p = 0.39), although mean value for ecotype III was slightly higher than values for ecotypes I and II (table 1).

To study usefulness and sensitivity in quantifying siphonal variability, for each ecotype 10 indices coefficient of variation (CV) were calculated. Thus, within each ecotype CV values were compared, revealing that CV values for all nine siphon indices were similar (ranged from 7.95% to 9.81%, 10.00% to 11.85% and 7.11% to 9.90% for I, II and III ecotypes, respectively), with the exception of WI. Lower values of CV for WI were obtained; there were 5.47%, 6.10% and 5.08% for I, II and III ecotypes, respectively (figure 2). Since, all siphonal indices (SI1-SI9) have equivalent sensitivity and usefulness, we decided to use SI1 for further comparison and discussion. In our study mean value of SI1 ranges from 4.73 (EI), 4.85 (EII) to 5.49 (EIII), which was slightly higher than previously published data, with SI values from 3.08 to 5.14 recorded for biotype *molestus* and from 4.4 to 6.4 for biotype *pipiens* (Jobling, 1938; Eritja and Aranda, 1995; Vinogradova *et al.*, 1996; Vinogradova and Ivnitsky, 2009), or about 4.5 or less for *molestus* and about 5.0 or more for *pipiens* (Marshall, 1944; Gutsevich *et al.*, 1974). Discordance of SI values may be due to the influence of different factors, primarily the use of exuviae, not the larvae, the measurement without permanent mounting and environmental conditions. Although the use of exuviae can affect the results because of the flexibility in the shape, there is no evidence that this happened here, especially as they not permanently mounted. This method has the advantage over the use of larvae, because it allows the application of different methods for larvae and adult individuals and thus allows obtaining different types of data for a particular individual.

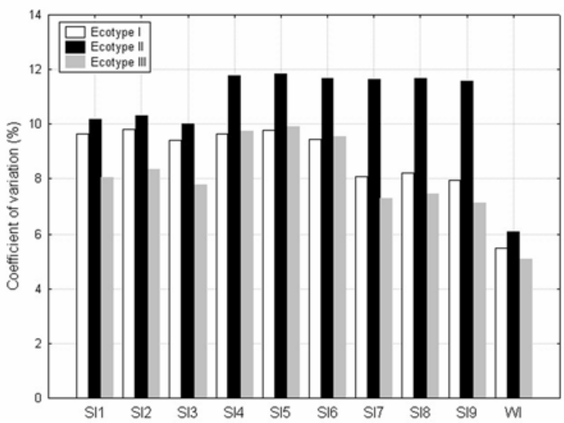

Figure 2. Coefficient of variation of 9 siphonal indices (SI1-SI9) and one width index (WI) of "urban" (I and II) and "rural" (III) ecotypes of *C. pipiens* from Novi Sad.

It is important to highlight that in many mosquito species factors influencing differentiation of populations and species act at the larval stage, following morphological, physiological and behavioural adaptations of the adult stage (Ivnitsky, 1994; Vinogradova and Ivnitsky, 2009). In addition, it was suggested that niche-specific selection, related to the specificity of larval feeding, determined the exploration of different trophic niches and types of aquatic biotopes (Ivnitsky, 1994). Therefore, morphological constraints imposed by a highly specialized ecological niche, such as urban habitats, influence the divergence of certain morphological characters between "urban" and "rural" forms (Vinogradova and Ivnitsky, 2009). Although the siphon is not directly involved in the feeding process, siphon characters, such as SI, show divergence among populations of *C. pipiens* and can be used for their separation (Vinogradova *et al.*, 1996). Results presented herein showing that differences between "urban" (I and II) and "rural" (III) ecotypes are in concordance with published data (Petrarca *et al.*, 1980; Sabatinelli and Petrarca, 1980; Eritja and Aranda, 1995). Since both *C. pipiens* forms feed on avian and mammalian hosts (including humans) they are considered a bridge-vector for the transmission of arboviruses (such as West Nile virus) from hosts to humans (Kilpatrick *et al.*, 2007; Reusken *et al.*, 2010). Therefore, precise identification of such taxa of epidemiological importance is of great significance for designing vector control strategies. Beyond of this study, differences between biotypes will be fully tested by other larval (number of pectin spines and number of siphonal seta) and adult (wing measurements) morphometric parameters and genetic data using more discriminating methods (geometric morphometrics of siphon and wings and protein electrophoresis) (Krtinić B., Ludoški J., Milankov V., unpublished).

Acknowledgements

B. K. is supported by Ciklonizacija d.o.o. Novi Sad. J. L. and V. M. are supported in part by the Ministry of Science of Serbia (Dynamics of gene pool, genetic and phenotypic variability of populations, determined by the environmental changes, No. 173012), and the Provincial Secretariat for Science and Technological Development (Molecular and phenotypic diversity of taxa of economical and epidemiological importance, and endangered and endemic species in Europe).

References

BAHNCK C. M., FONSECA D. M., 2006.- Rapid assay to identify the two genetic forms of *Culex* (*Culex*) *pipiens* L. (Diptera: Culicidae) and hybrid populations.- *American Journal of Tropical Medicine and Hygiene*, 75: 251-255.

BECKER N., JÖST A., STORCH V., WEITZEL T., 1999.- Exploiting the biology of urban mosquitoes for their control.- *Journal of the American Mosquito Control Association*, 25 (1): 6-17.

BECKER N., PETRIĆ D., ZGOMBA M., BOASE C., DAHL C., LANE J., KAISER A., 2010.- *Mosquitoes and their control*. 2nd edition.- Springer Verlag, Berlin Heidelberg, Germany.

BELKIN N. J., 1962.- *The mosquitoes of the South Pacific (Diptera: Culicidae)*.- University of California Press, Berkley, USA.

BOŽIČIĆ B., 1985.- *The mosquitoes (Diptera: Culicidae) of the Fruška Gora Mountain. Monographs of Fruška Gora*.- Matica srpska, Novi Sad, Serbia. (in Serbian)

BROGDON W. G., 1981.- Use of the siphonal index to separate *Culex pipiens* subspecies and hybrids.- *Mosquito Systematics*, 13: 129-137.

BROGDON W. G., 1984.- The siphonal index I. A method for evaluating *Culex pipiens* subspecies and intermediates.- *Mosquito Systematics*, 16: 144-152.

BYRNE K., NICHOLS R. A. 1999.- *Culex pipiens* in London underground tunnels: differentiation between surface and subterranean population.- *Heredity*, 82: 7-15.

CHEVILLION C., ERITJA R., PASTEUR N., RAYMOND M., 1993.- Commensalism adaption and gene flow: mosquitoes from the *Culex pipiens* complex in different habitats.- *Genetic Research*, 66: 147-157.

CHEVILLON C., RIVET Y., RAYMOND M., ROUSSET F., SMOUSE P. E., PASTEUR N., 1998.- Migration/selection balance and ecotypic differentiation in the mosquito *Culex pipiens*.- *Molecular Ecology*, 7: 197-208.

CORNEL A. J., MCABEE R. D., RASGON J., STANICH M. A., SCOTT T. W., COETZEE M., 2003.- Differences in extent of genetic introgression between sympatric *Culex pipiens* and *Culex quinquefasciatus* (Diptera: Culicidae) in California and South Africa.- *Journal of Medical Entomology*, 40 (1): 36-51.

CRANSTON P. S., RAMSDALE C. D., SNOW K. R., WHITE G. B., 1987.- Keys to the adults, male hypopigia, fourth-instar larvae and pupae of the British mosquitoes (Culicidae) with notes on their ecology and medical importance.- *Freshwater Biological Association Scientific Publication*, 48: 1-152.

DAHL C., 1988.- Taxonomic studies on *Culex pipiens* and *C. torrentium*, pp. 149-175. In: *Biosystematics of haematophagous insects* (SERVICE M.W., Ed.).- Clarendon Press, Oxford, UK.

ERITJA R., ARANDA K., 1995.- Preliminary observations on sex-related variation in morphological character of *Culex pipiens* (Diptera: Culicidae) larvae in northeastern Spain.- *Mosquito Systematics*, 27 (2): 73-77.

FONSECA D. M., KEYGHOBADI N., MALCOLM C. A., MEHMET C., SCHAFFNER F., MOGI M., FLEISCHER R. C.,WILKERSON R. C., 2004.- Emerging vectors in the *Culex pipiens* complex.- *Science*, 303: 1535-1538.

GOMES B., SOUSA C. A., NOVO M. T., FREITAS F. B., ALVE, R., CÔRTE-REAL A. R., SALGUEIRO P., DONNELLY M. J., ALMEIDA A. P. G., PINTO J., 2009.- Asymmetric introgression between sympatric molestus and pipiens forms of *Culex pipiens* (Diptera: Culicidae) in the Comporta region, Portugal.- *BMC Evolutionary Biology*, 9: 262.

GUTSEVICH A.V., MONCHADSKII A. S., SHTAKEL'BERG A. A., 1974.- *Fauna of the USSR Diptera*. Vol. 3, No. 4. *Mosquitoes family Culicidae*.- Keter Publishing House Jerusalem Ltd., Jerusalem, Israel.

HAMER G. L., KITRON U. D., BRAWN J. D., LOSS S. R., RUIZ M. O., GOLDBERG T. L., WALKER E. D., 2008.- *Culex pipiens* (Diptera: Culicidae): a bridge vector of West Nile to humans.- *Journal of Medical Entomology*, 45 (1): 125-128.

HARBACH R. E., KNIGHT K. L., 1980.- *Taxonomists' glossary of mosquito anatomy*.- Plexus Publishing Inc., Marlton, New Jersey, USA.

HARBACH R. E., HARRISON B. A., GAD A. M., 1984.- *Culex* (*Culex*) *molestus* Forskål (Diptera: Culicidae): neotype, designation, description, variation, and taxonomic status.- *Proceedings of the Entomological Society of Washington*, 86: 521-542.

HARBACH R. E., DAHL C., WHITE G. B., 1985.- *Culex* (*Culex*) *pipiens* Linnaeus (Diptera: Culicidae): concepts, type designations and description.- *Proceedings of the Entomological Society of Washington*, 87: 1-24.

ISHII T., 1980.- On the *Culex pipiens* group in Japan. III. A historical review of its research.- *Journal of Science University of Tokushima*, 13: 29-62.

IVNITSKY S. B., 1994.- Transformation of quantitative characters during divergence in the genus *Aedes* (Diptera: Culicidae).- *Zoologicheskij Zhurnal*, 73 (12): 71-80.

JOBLING B., 1938.- On two subspecies of *Culex pipiens* L. (Diptera).- *Transactions of the Royal Entomological Society of London*, 87: 193-216.

JUPP P. G., 1978.- *Culex* (*Culex*) *pipiens pipiens* Linnaeus and *Culex* (*Culex*) *pipiens quinquefasciatus* Say in South Africa: morphological and reproductive evidence in favour of their status as two species.- *Mosquito Systematics*, 10: 461-473.

KENT R. J., HARRINGTON L. C., NORRIS D. E., 2007.- Genetic differences between *Culex pipiens f. molestus* and *Culex pipiens pipiens* (Diptera: Culicidae) in New York.- *Journal of Medical Entomology*, 44: 50-59.

KILPATRICK M., KRAMER L. D., JONES M. J., MARRA P. P., DASZAK P., FONSECA D. M., 2007.- Genetic influences on mosquito feeding behavior and the emergence of zoonotic pathogens.- *American Journal of Tropical Medicine and Hygiene*, 77: 667-671.

KOTHERA L., GODSEY M., MUTEBI J. P., SAVAGE H. M., 2010.- A comparison of aboveground and belowground populations of *Culex pipiens* (Diptera: Culicidae) mosquitoes in Chicago, Illinois, and New York City, New York, using microsatellites.- *Journal of Medical Entomology*, 47 (5): 805-813.

KRUPPA T. R., 1987.- Morphometric studies of different strains of *Culex pipiens* s.l. (Diptera: Culicidae).- *Akaieka Newsletter*, 11 (2): 4-11.

MARSHALL J., 1944.- The morphology and biology of *Culex molestus*: observational notes for investigators.- *The British Mosquito Control Institute*, 34: 1-14.

MCMILLAN M. L., 1958.- Study of a naturally occurring population intermediate between *Culex pipiens pipiens* and *Culex pipiens quinquefasciatus*.- *American Journal of Tropical Medicine and Hygiene*, 7: 505-511.

PETRARCA V., SABATINELLI G., COLUZZI M., 1980.- Significance of some biometric differences in different populations of the complex *Culex pipiens* (Diptera: Culicidae).- *Parassitologia*, 22: 340-342. (in Italian)

RASGON J. L., CORNEL A. J., SCOTT T. W., 2006.- Evolutionary history of a mosquito endosymbiont revealed through mitochondrial hitchhiking.- *Proceedings of Royal Society B*, 273: 1603-1611.

REUSKEN C. B., DE VRIES A., BUIJS J., BRAKS M. A., DEN HARTOG W., SCHOLTE E.-J., 2010.- First evidence for presence of *Culex pipiens* biotype *molestus* in the Netherlands, and of hybrid biotype *pipiens* and *molestus* in northern Europe.- *Journal of Vector Ecology*, 35: 210-212.

RIBEIRO H., PIRES C. A., RAMOS H. C., CAPELA R. A., 1983.- Research on the mosquitoes of Portugal (Diptera, Culicidae). VIII- On the occurrence of *Culex* (*Culex*) *molestus* Forskål, 1775.- *Journal Sociedade Ciencias Medicas Lisboa*, 147: 185-188.

SABATINELLI G., PETRARCA V., 1980.- Analysis of some morphological differences of the complex *Culex pipiens* (Diptera: Culicidae).- *Atti XII Congresso Nazionale Italiano di Entomologia*, 2: 387-389. (in Italian)

SERVICE M. W., 1968.- The taxonomy and biology of two sympatric sibling species of *Culex*, *C. pipiens* and *C. torrentium* (Diptera, Culicidae).- *Journal of Zoology*, 156: 313-323.

SMITH J. L., KEYGHOBADI N., MATRONE M. A., ESCHER R. L., FONSECA D. M., 2005.- Cross-species comparison of microsatellite loci in the *Culex pipiens* complex and beyond.- *Molecular Ecology Notes*, 5: 697-700.

SPIELMAN A., 1967.- Population structure in the *Culex pipiens* complex of mosquitoes.- *Bulletin of the World Health Organization*, 37: 271-276.

SPIELMAN A., 2001.- Structure and seasonality of nearctic *Culex pipiens* populations.- *Annals of the New York Academy of Sciences*, 95: 220-234.

TABACHNIK W. J., BLACK W. C., 1995.- Making a case for molecular population genetic studies of arthropod vectors.- *Parasitology Today*, 11: 27-30.

VINOGRADOVA E. B., 2003.- Ecophysiological and morphological variations in mosquitoes of the *Culex pipiens* complex (Diptera: Culicidae).- *Acta Societas Zoologicae Bohemicae*, 67: 41-50.

VINOGRADOVA E. B., IVNITSKY S. B., 2009.- Variability of quantitative morphological traits of mosquito larvae in some species of the *Culex pipiens* complex (Diptera: Culicidae).- *Entomological Review*, 4: 390-398.

VINOGRADOVA E. B., SHAIKEVICH E. V., 2007.- Morphometric, physiological and molecular characteristics of underground populations of the urban mosquito *Culex pipiens* Linnaeus f. *molestus* Forskål (Diptera: Culicidae) from several areas of Russia.- *European Mosquito Bulletin*, 22: 17-24.

VINOGRADOVA E. B., REZNIK S. Y., KUPRIJANOVA E. S., 1996.- Ecological and geographical variations in the siphonal index of *Culex pipiens* larvae (Diptera: Culicidae).- *Bulletin of Entomological Research*, 86: 281-287.

WEITZEL T., COLLADO A., JÖST A., PIETSCH K., STORCH V., BECKER, N., 2009.- Genetic differentiation of populations within the *Culex pipiens* complex and phylogeny of related species.- *Journal of the American Mosquito Control Association*, 25 (1): 6-17.

Authors' addresses: Vesna MILANKOV (corresponding author, vesna.milankov@dbe.uns.ac.rs,), Jasmina LUDOŠKI, Faculty of Sciences, Department of Biology and Ecology, University of Novi Sad, Trg Dositeja Obradovića 2, 21000 Novi Sad, Serbia; Bosiljka KRTINIĆ, Ciklonizacija, Primorska 76, 21000 Novi Sad, Serbia.

Received March 26, 2011. Accepted November 29, 2011.