ENVIRONMENTAL ASSESSMENT OF HEALTH INDICATORS OF WOODY PLANTS IN NATURAL AND MAN-MADE CONDITIONS

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Abstract. The indicators of the sanitary condition of woody plants in the conditions of natural and artificial ecosystems of the Voronezh and Saratov Regions were established and analyzed. In suburban ecosystems of the research area, the state of woody plants is determined at the level of weak damage. In suburban areas of Balashov with the greatest economic and technosphere potential, woody plants reach an average (moderate) degree of damage. In remote relatively preserved ecosystems (control parameters), the state of these organisms corresponds to a weak ecological diagnostic criterion. According to the identified average arithmetic sanitary indicators in the Voronezh Region, the best condition is characterized by woody plants in the ecosystems of Novohopersk and Gribanovskiy, in the Saratov Region – in Turki and Samoylovka. There were also significant differences in the arithmetic mean parameters of the sanitary condition of this group plants representatives differentiated by functional zones. The highest damage values are typical for woody plants in the urban ecosystems of industrial zones. The maximum danger to woody plants was observed within these zones in Povorino, Borisoglebsk, and Balashov with high-level average values of damage to these organisms. Significant concern is also caused by the defeat of the analyzed group of organisms in these cities as part of the ecosystems of the settlement zones. The results of the assessment of the sanitary condition of woody plants should be used as a scientific basis for landscaping, environmental protection and reforestation. Measures are proposed to improve the condition of woody plants in various types of ecosystems in the research area. According to the sanitary state of these organisms, it is possible to determine the limits of sustainability of ecological frameworks and differentiate the parameters of environmental comfort for the local population in different territorial categories.

Keywords: municipal districts of Voronezh Region; municipal districts of Saratov Region; natural ecosystems; artificial ecosystems; recreation zones; settlement zones; industrial zones; sanitary condition of woody plants; values and levels of plant degradation; territorial differences in plant viability; trends in plant biological stability; environmental quality; values of environmental comfort for residents; relevance of measures for plant protection.

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LESS LEGS MORPHOMETRIC CHARACTERS OF THE DOLICHOPUS LATREILLE SPECIES, 1796 (DIPTERA, DOLICHOPODIDAE)

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Abstract. A comparative analysis of 30 species of the Dolichopodidae family in a phylogenetic context was conducted to examine interspecific variation in the legs morphometry. Five relative traits of legs from 12 and seven absolute traits from nine showed significant phylogenetic signal. A set of traits, such as relatively short hind tibia and relatively long fore and middle tibia and the first segments of the hind legs allowed to allocate Dolichopus species from the other ones. The projection of the phylogenetic tree of Dolichopus species into the morphospace allowed us to divide it into four individual areas: not closely related species, but species having similar modifications of males’ legs tended to cluster. This suggests that the legs morphometric traits should be mainly under pressure of sexual selection. It has also been revealed that the elongation of the first segment of hind tarsi in Dolichopus species is associ-
Introduction

Morphometric methods linked with DNA-based molecular genetic analysis represent a powerful new approach for taxonomic issues resolving and evolutionary process study [1, p. 11].

Ornamented legs are commonplace in the Dolichopodidae and they, as shown for certain species, can be used in courtship or in male–male interactions. Such traits are widely used for taxonomic identification of the species [2, p. 146] and classification of their functional significance can be found in the literature [3, p. 145]. For instance, male Neurigona quadrifasciata (Fabricius, 1781) demonstrates its plumed fore tarsi to female, approaching from the rear [4, p. 32]. Male Hydrophorus praeos (Lehmann, 1822) uses spines on femora to keep riding a female [5, p. 14] Male Dolichopus plumipes (Scopoli, 1763) uses bilateral feathering first segment of middle tarsi in courtship as follows. Male approaching female and vibrating with its wings slowly raises the middle legs parallel to the abdomen and stretches them horizontally [6, p. 43]. However, for most species legs modifications are not well understood yet.

One less explored area is morphometric characters of legs, although sometimes differences in sizes of legs segments are used for identification species (for example, in the genera Argyra Macquart, 1834, Campsicnemus Haliday, 1851, Tachytrechus Haliday, 1851) [2, p. 190]. To investigate differences between species in legs morphometric characters and to indicate evolutionary trends a detailed comparative analyses is required.

Species of the genus Dolichopus Latreille, 1796 represent a suitable model group for morphometric traits variation study for the following reasons: firstly, according to recent studies based on molecular and morphological traits, it is a single evolutionary unit [7, p. 21; 8, p. 310], and, secondly, leg modifications in males of this genus are diverse: there are extensions [85] and ornamentations (Dolichopus plumipes (Scopoli, 1763)).

Thus, the purpose of this paper is a comparative study of the legs morphometric diversity of Dolichopodidae species and the estimation of their phylogenetic signal by molecular phylogeny mapping.

Material and methods

This study is based on 1240 specimens from 21 species of the Dolichopus genus. Nine species belonging to Dolichopodinae, Diaphorinae and Symphyccinae subfamilies were chosen as outgroup (table 1). Males of eleven species from 21 studied ones had obvious leg modifications. The material was obtained from the collections of Ecology and Zoology of Invertebrates Department, Voronezh State University.

The legs were separated from the body, placed on a microscope slide under a cover slip and photographed with Levenhuk C310 NG microscope digital camera. To eliminate digitizing error, all wings were digitized two times. Measurement repeatability was very high.

Nine traits were measured using ImageJ software: length of fore, middle and hind femora (F1, F2, F3), length of fore, middle and hind tibia (T1, T2, T3) and length of the first segment of fore, middle and hind tarsi (tar1, tar2, tar3) as well. Then twenty relative characters were calculated: the ratios of fore femora and fore tibia (F1/T1), fore femora and first segment of fore tarsi (F1/tar1), fore femora and middle femora (F1/F2), fore femora and hind femora (F1/F3), fore tibia and the first segment of fore tarsi (T1/tar1), fore tibia and middle tibia (T1/T2), fore tibia and hind tibia (T1/T3), middle femora and middle tibia (F2/T2), middle femora and the first segment of middle tarsi (F2/tar2), hind femora and hind tibia (F3/T3), hind femora and the first segment of hind tarsi (F3/tar3).

A multivariate analysis of variance (MANOVA) and post-hoc Tukey’s test were performed to examine differences represented among species, sexes, and sides (left and right legs). All statistical analyses were made with the Statistica software [9].

Phylogenetic relationships among the species were derived from an analysis of mitochondrial gene sequences – the cytochrome oxidase subunit I (COI) gene (810 characters), previously submitted to GenBank [10; 11, p 455; 12, p. 605]. Suitability of COI gene for resolving phylogenetic relationships has been repeatedly confirmed for a wide range of insects’ taxa [13, p. 2; 14, p. 566].

Sequences from GenBank were aligned using ClustalW software [15]. The phylogenetic reconstruction was provided with maximum parsimony analysis in MEGA software [16]. The significance of the inner branching pattern was estimated by a bootstrap analysis with 1000 pseudo-replicates.

As a measure of phylogenetic signal of legs morphometric characters, we used Pagel’s lambda (λ) [17, p. 680]. A Pagel’s lambda value close to 1 indicates the presence of an explicit phylogenetic signal, while a value closer to 0 indicates the absence of a phylogenetic signal of the trait [18, p. 714]. To calculate Pagel’s lambda, the picante package [19] was used in R environment [20].

For testing purpose the indications of differences of the metric from 0, a p-value was obtained by randomizing the trait data 999 times. The P value was calculated as the proportion of cases the value of λ for randomized trait data exceeded the real data means [21].

Principal Component Analysis (PCA) was performed on the legs morphometric traits to detect and to describe the differences among taxa. The Pearson correlation coefficient was applied to reveal a set of interdependent characters. For this objective, the dataset of Procrustes Coordinates, describing the wing shape of Dolichopodidae species and obtained using geometric morphometrics methods (more detailed described in [22]) was used. The nomenclature of wing venation carried out as per Grichanov and Brooks [23, p. 1288].

Keywords: Diptera; Dolichopodidae; Dolichopus; legs morphometric characters; morphological characters; femora; tibia; tarsi; sexual dimorphism; wing shape; modification of legs; modification of tibia; modification of tarsi; COI gene; morphometry; ornaments; phylogenetic signal; morphometric characters; nomenclature of wing venation carr; phylogeny; phylogenetic tree.
Results of MANOVA indicated legs morphometric traits significant differences among species (table 2), but not between sides. Sexes showed significantly lower differences in legs morphology in comparisons with species. The most significant effect of «species×sex» interaction suggests that there should be differences in the patterns of sexual dimorphism among species. The results of post-hoc Tukey’s test indicate that the difference between the largest numbers of species are observed in the following relative traits: F1/tar1, T1/tar1, F2/T2. The lowest degree of variation was observed in F1/F2.

Five relative traits from 12 ones showed a high phylogenetic signal, both males and females (table 3). Length of the first segments of fore and middle tarsi hardly plumate indicates the lowest phylogenetic signal for any studied traits.

Based on PCA results, performed on morphometric data, the position of all species in the morphospace mainly was determined by the two of the first principle components, accounted more than 98% of the total variance. The first principle component (PC1), explaining the most proportion of variance (85.83%), was associated with changes in the length of T3, T2, and T1. The second principal component (PC2), representing 12% of total variation, reflects changes in the length of tar3.

The mapping of the phylogenetic tree into the morphospace indicated that along PC1 species tended to divide into two clusters: Dolichopus species (specimens with shortened T3 and elongated T1 and T2), that were clustered together in the area of negative values of PC1, and the outgroup species (specimens with elongated T3 and shortened T1 and T2). The PC2 axis described a variation from a relatively short tar3 to a little long one (from Poecilobothrus regalis to Dolichopus claviger) (fig. 1).

Only one relative trait was found with a statistically significant amount of phylogenetic signal in the leg morphology of Dolichopus species (without outgroup) – T1/T2 (λ = 0.755, P = 0.07). In this case, PCA also indicated the fact most legs variation was concentrated in two dimensions. The PC1 and PC2 are calculated for the two dimensions. The PC1 and PC2 accounted about 84% of total variance. The PC1 explained 65.89% of the total variations and described a variation from Dolichopus claviger (species with elongated tar3 and shorter T1) to Dolichopus plumipes and D. linears (species with shorted tar3 and elongated T1). The PC2 explained 19% of the total variations and associated with separation among species with shorted T2 length (Dolichopus longicornis) and with elongated T2 ones (D. plumipes) (fig. 2). A permutation test for the hypothesis of phylogenetic signal absence as for the legs morphometric data indicates the probability of finding a shorter tree on level P < 0.0001, which demonstrates the presence of a significant phylogenetic signal in the studied traits.

| № | Species | Specimens | Males ornamented legs |
|---|---------|-----------|-----------------------|
| 1 | Dolichopus acuticornis Wiedemann, 1817 | 20 ♂, 46 ♀ | without modification |
| 2 | Dolichopus arborustorum Zetterstedt, 1843 | 20 ♂, 20 ♀ | without modification |
| 3 | Dolichopus argyroptarsis Wahlberg, 1850 | 12 ♂, 2 ♀ | 3rd–5th segments of middle tarsus silvery-white, slightly enlarged |
| 4 | Dolichopus brevipennis Meigen, 1824 | 18 ♂, 16 ♀ | 5th segments of fore tarsus enlarged |
| 5 | Dolichopus campestris Meigen, 1824 | 22 ♂, 24 ♀ | without modification |
| 6 | Dolichopus cliferomoratus Macquart, 1827 | 30 ♂, 32 ♀ | segments of fore tarsus curved, covered with erected hairs |
| 7 | Dolichopus claviger Stannius, 1831 | 20 ♂, 16 ♀ | 5th segments of fore tarsus enlarged |
| 8 | Dolichopus discifer Stannius, 1831 | 18 ♂, 14 ♀ | 5th segments of fore tarsus enlarged |
| 9 | Dolichopus latilimbatus Macquart, 1827 | 50 ♂, 46 ♀ | without modification |
| 10 | Dolichopus lepidus Staeger, 1842 | 50 ♂, 41 ♀ | hind tibia thickened |
| 11 | Dolichopus linearis Meigen, 1824 | 24 ♂, 20 ♀ | without modification |
| 12 | Dolichopus longicornis Stannius, 1831 | 32 ♂, 52 ♀ | without modification |
| 13 | Dolichopus longitarsis Stannius, 1831 | 50 ♂, 79 ♀ | hind tibia thickened |
| 14 | Dolichopus meigeni Loew, 1857 | 12 ♂, 2 ♀ | without modification |
| 15 | Dolichopus migrans Zetterstedt, 1843 | 18 ♂, 20 ♀ | 5th segments of fore tarsus enlarged |
| 16 | Dolichopus pennaatus Meigen, 1824 | 50 ♂, 38 ♀ | 1st–2nd segments of middle tarsus hardly plumate, 4th–5th segments silvery-white |
| 17 | Dolichopus plumipes (Scopoli, 1763) | 54 ♂, 14 ♀ | Middle tibia thin and 1st segment of middle tarsi hardly plumate |
| 18 | Dolichopus remipes Wahlberg, 1839 | 6 ♂, 30 ♀ | 3rd and 4th segments of hind tarsi plumate |
| 19 | Dolichopus ringdahlii Stackelberg, 1930 | 36 ♂, 14 ♀ | without modification |
| 20 | Dolichopus simplex Meigen, 1824 | 40 ♂, 40 ♀ | without modification |
| 21 | Dolichopus ungulatus (Linneaus, 1758) | 50 ♂, 42 ♀ | without modification |

Outgroup

| № | Species | Specimens | Males ornamented legs |
|---|---------|-----------|-----------------------|
| 22 | Poecilobothrus chrysocygogos (Wiedemann, 1817) | 28 ♂, 4 ♀ | 1st–2nd segments of fore tarsus with white rings |
| 23 | Poecilobothrus regalis (Meigen, 1824) | 24 ♂, 50 ♀ | without modification |
| 24 | Ethiromyia chalybea (Wiedemann, 1817) | 10 ♂, 4 ♀ | without modification |
| 25 | Sybistroma binodicornis Stackelberg, 1941 | 14 ♂, 46 ♀ | without modification |
| 26 | Sybistroma crimpes Staeber, 1842 | 20 ♂, 20 ♀ | without modification |
| 27 | Argyra diaphana (Fabricius, 1775) | 22 ♂, 40 ♀ | without modification |
| 28 | Argyra leucocephala (Meigen, 1824) | 11 ♂, 16 ♀ | without modification |
| 29 | Chrysotus cilipes Meigen, 1824 | 6 ♂, 54 ♀ | without modification |
| 30 | Sympecus pulcarius (Fallen, 1823) | 30 ♂, 110 ♀ | without modification |
As for legs morphology, the projection of the phylogeny into the morphospace of *Dolichopus* species showed a possibility to identify it for individual areas. The first area, located in the upper right corner, presented males with enlarged or plumage segments of middle legs (*Dolichopus plumipes*, *D. pennatus* and *D. argyrotarsis*). The second area is located in the morphospace in the zone of negative PC1 values, it is characterized by the largest spread. It includes the species, the males of which have modified segments of the forelegs (*Dolichopus brevipennis*, *D. discifer*, *D. claviger* and *D. cili-femoratus*). The third area positioned in the central part of the diagram presented species with modifications of the hind legs (*Dolichopus lepidus*, *D. longitarsis* и *D. remipes*). The species with long apicoventral bristle on fore tibia tended to be clustered in the lower right corner.

### Table 2 – The results of MANOVA of 12 relative legs morphometric traits for 30 dolichopodid species

| Effect               | Wilk’s lambda | F    | Effect df | Error df | P        |
|----------------------|---------------|------|-----------|----------|----------|
| Species              | 0.0001        | 59.0 | 336       | 15653    | <0.00001 |
| Sex                  | 0.6866        | 10.7 | 12        | 1439     | 0.001    |
| Species×sex          | 0.0598        | 13.7 | 324       | 15257    | <0.00001 |
| Side                 | 0.9934        | 1    | 12        | 1410     | 0.67     |

### Table 3 – Pagel’s lambda value and *P* values, calculated from 999 randomizations, of twenty relative and nine absolute traits of legs morphometry in 27 Dolichopodidae species

| Trait          | Both sexes | Female | Male |
|----------------|------------|--------|------|
|                | λ           |        |      |
|                | *P*         |        |      |
|                | *P*         |        |      |
|                | *P*         |        |      |
|                | *P*         |        |      |
|                | *P*         |        |      |
|                | *P*         |        |      |
|                | *P*         |        |      |
|                | *P*         |        |      |
|                | *P*         |        |      |
|                | *P*         |        |      |
|                | *P*         |        |      |
|                | *P*         |        |      |

*Note. λ – Pagel’s lambda, *P* – *P* value. Asterisks (*) mark traits showed statistically significant phylogenetic signal.*

*Figure 1 – Mapping of the legs morphometric trait variation of 30 Dolichopodidae species on the phylogenetic tree*
Spearman rank correlation coefficient (between legs morphometric traits and Procrustes coordinates of nine landmarks among 27 Dolichopodidae species) indicates a strong link between the length of tar3 with the displacement of the insertion point of dm-m with M1 (rs = –0.59, P < 0.05) and lengths of tar3 and R4+5 (rs = –0.65, P < 0.05). Spearman rank correlation coefficient also demonstrates a moderate relationship level between the length of T1 and the presence of the long apicoventral bristle (rs = 0.58, P < 0.05).

**Discussion**

The recent studies of morphologic characters of insects are focused on the wing shape as follow: discrimination of cryptic species [24–26], evaluation of developmental instability [27; 28], identification of both geographical and ecological variation [29–31] and evolutionary transitions [32]; so there are not many kinds of research on legs morphometry field.

As an example, a comparative analysis of morphometric characters of two dipteran species indicates that change patterns in the body shape associated with sexual dimorphism had a similar character for two non-closely related species (Prochyliza xanthostoma and Telostylinus angusticollis). The elongation of antennae and legs was associated with a decrease of head width and wing length, but the functional significance of these relationships remains uncertain [33, p. 609]. Moreover, based on the study of Titmus and Badcock [34, p. 535], the anterior segment of the tarsi became shorter in individuals Benthalia carbonaria (Meigen, 1804) (Chironomidae) infected by parasitic nematodes of the Mermitid family.

We have already tried to find a diagnostic significance of legs morphometric characters [35]; however, the present study is the first of the kind with a comparative analysis of variations in legs morphometric traits with the phyletic relationships between species. Our data indicate that several legs morphometric traits tend to have high phylogenetic signal.

A set of traits, such as relatively short hind tibia and relatively long fore and middle tibia and the first segments of the hind legs, allowed to allocate Dolichopus species from the outgroup ones. A clear sign of Dolichopus species is consistent with the molecular data indicating the genus as a monophyletic group as well as an independent evolutionary unit [8, p. 310].

It has also been revealed that the elongation of the first segment of hind tarsi in Dolichopus species is associated with the distal displacement of the insertion point of dm-m with M4 and the decrease of the length of R4+5. The functional hypothesis forwarded to explain the relationship of these traits should be ground on further searches of the wing shape variability in the family.

The position of the insertion point of dm-m with M4 indicates the most variance among dolichopodid species. This fact may prove the importance of its position for certain flight characteristics of the wing to be created. Furthermore, there are no significant differences in the position of the R4+5 apex among the representatives of the family [22, p. 697]. In addition, it is obvious that the increase of the length of the first segment of hind tarsi in Dolichopus species is combined with the presence of one or more strong dorsal setae on it. The last character is no more found inside the family Dolichopodidae, except for some species, for example, Poecilobothrus regalis (Meigen, 1824).

As for field observations, dolichoidid flies use hind tarsi to clean the wings’ membranes. The shortening of hind tibia (in combination with the elongation of the first segment of hind tarsi and the appearance of strong dorsal
bristles on it) may be some adaptation for more efficient wing cleaning and for improved flight characteristics as well.

The elongation of fore tibia in some species of Dolichopus (D. latilimbatus, D. linearis, D. longicornis) correlated with the presence of a long apicoventral bristle. This fact was demonstrated by the Spearman’s rank correlation coefficient and greater proximity of these species in the morphospace. However, it is difficult to say about the functional purpose of this combination of traits at this stage; further research on the behavioral traits associated with this bristle of dolichopodid flies is needed for it.

A significant interspecific variation in legs morphology of Dolichopus species was demonstrated with the PCA and indicate a variation of the lengths of the first segment of hind legs and the middle and hind tibia. Dolichopus claviger and D. plumipes keep extreme positions in morphospace. The males of these species are characterized by obvious leg modifications: the thin middle tibia and the densely feathered first segment of the middle tarsi of Dolichopus plumipes and an enlarged black fifth segment of fore tarsi of Dolichopus claviger. Species with the males without obvious modifications of the legs have an isolated location in the morphospace.

Having considered the distribution of species, we conclude that the similarity of legs morphology in studied Dolichopus species is not always the result of common origin. Dolichopus longicornis and D. acuticornis is the only example in our study – closely related species were close to each other in the morphospace. In other cases, the distribution of species leads to the conclusion for morphometric similarity caused by a similar pressure of selection. This is confirmed by the selection on the plot of clusters with not closely related species, but with species having similar modifications of males’ legs. Whereas, clusters allocated on the basis of molecular data are both monomorphic and dimorphic species (for instance, Dolichopus plumipes and D. simplex, D. brevipennis and D. angulatus).

The most parsimonious phylogenetic tree indicates that the monomorphism is a plesiomorphic state for the family, as well as the presence of several parallel episodes of the occurrence of similar leg modifications (for example, the appearance of the enlarged fifth segment of fore tarsi in Dolichopus claviger and D. discifer). The total morphology of the legs seems to be depended on the modifications of the males’ legs as it was documented that some Dolichopus male species used modified legs to attract females and compete for their attention with other males [6, p. 43], it should be concluded that the legs morphometric traits are mainly under pressure of sexual selection.

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Legs morphometric characters of the Dolichopus Latreille species, 1796 (Diptera, Dolichopodidae)

МОРФОМЕТРИЧЕСКИЕ ПРИЗНАКИ НОГ
ВИДОВ РОДА DOLICHOPUS LATREILLE, 1796 (DIPTERA, DOLICHOPODIDAE)

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Аннотация. Для оценки межвидовой изменчивости морфометрических признаков ног был проведен сравнительный анализ 30 видов из семейства Dolichopodidae с учетом их филогении. Пять из двенадцати относительных признаков ног и семь из девяти абсолютных продемонстрировали статистически значимый филогenetический сигнал. Были выделены комплексы признаков, которые позволяют отделять виды Dolichopus от видов внешней группы: это относительно короткие задние голени и относительно длинные передние и средние голени, а также удлинённый первый членик задней лапки. Проекция филогенетического дерева видов Dolichopus на морфоструктуре позволила разделить его на четыре области: были кластеризованы не близко родственные виды, а виды, имеющие сходные модификации ног самцов. Это говорит о том, что морфометрические признаки ног в основном находятся под давлением полового отбора. Также было выявлено, что удлинение первого сегмента задних лапок у видов Dolichopus связано с дистальным смещением точки слияния dm-t с M3 и уменьшением длины R5+6. Обсуждается функциональное значение этого сочетания признаков.

Ключевые слова: Diptera; Dolichopodidae; Dolichopus; морфометрия ног; украшения; филогенетический сигнал; морфометрические признаки; морфологические признаки; бёдра; голени; лапки; половой диморфизм; форма крыла; модификации ног; модификации голеней; модификации лапок; COI ген; филогения; филогенетическое дерево.