Do *Anguis fragilis* L. and *Anguis colchica* (Nordman, 1840) hybridize in the south central part of Poland? Morphological signs of introgression between two species of slow worm and range of *Anguis colchica* in Poland – preliminary results of research

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1. Introduction

Recent nuclear and mitochondrial DNA analysis revealed 5 clades within genus *Anguis* which elevated to the species level (Gvoždík et al., 2013). However, hybrid specimens between some of these taxa were detected (Gvoždík et al., 2013; Szabó and Vörös, 2014). Two slow worm species occur in Poland: *A. fragilis* and *A. colchica*. Still little is known with regard to the exact distribution of contact zone of these two forms (Skórzewski et al., 2012; Kaczmarek, 2015).

In our paper we show some evidence that support hypothesis of the occurrence of hybridization between two slow worm species.

2. Material and methods

Two sets of data were analyzed in the context of *A. fragilis*/*A. colchica* differentiation in Poland. The **first** contains the truss network measurements of the pileus surface (fig. 1 left) and meristic characters: number of scales around central part of body (SCR) and number of supralabial scales. Discriminant function analysis (DFA) was performed on values of meristic characters and log-transformed residuals from truss network distances regressed on head length. Individuals were divided into three groups: *A. fragilis* (western Poland), *A. colchica* (eastern Poland and Hungary) and eastern part of Opole Plain.

The **second** dataset contains "traditional" linear measurements of the head (fig. 1 right). Obtained data were log-transformed for DFA. In this case specimens were classified to species in the base of the value of SCR and visibility of ear opening. The specimens which exhibit intermediate type of morphology were considered as hybrids

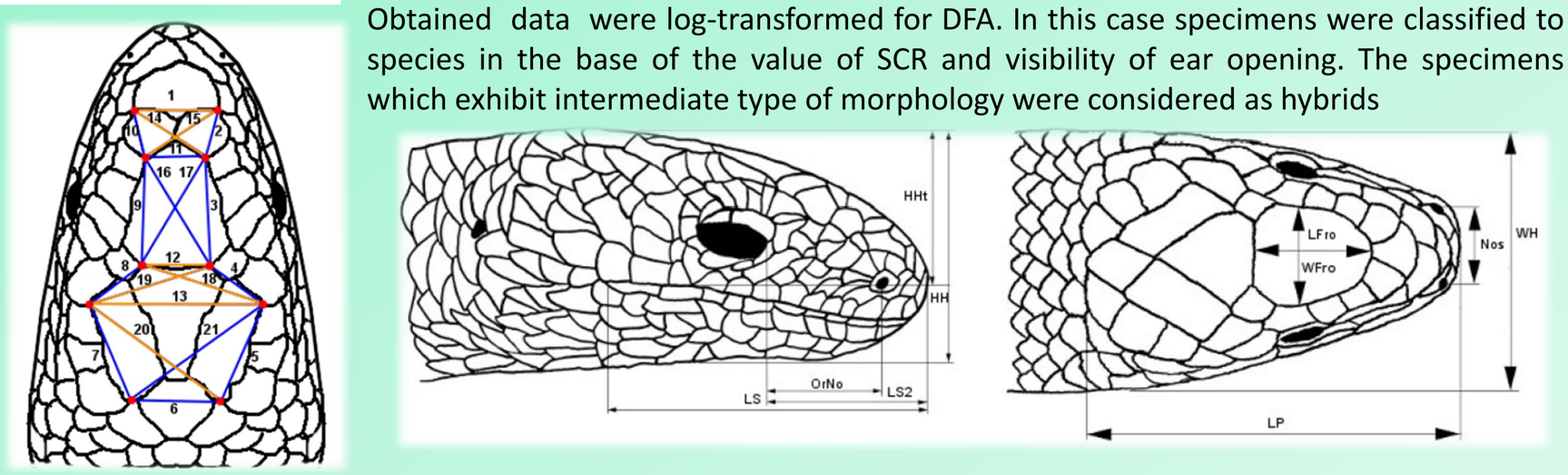

Fig. 1. Truss network distances of the pileus surface for the first analysis (left) and traditional linear measurements of the head for the second analysis (right). Truss network distances (left) which are the most correlate with the first canonical discriminant function and significantly discriminate groups are orange.

3. Results and discussion

DFA performed on the first dataset gave two canonical discriminant functions in which only the first is significant ($P < 0.001$). The second is insignificant ($P = 0.07$). The analysis revealed intermediate morphotype of individuals from Opole Plain (fig. 2 and fig. 4) It should be noted that meristic characters are the most correlate with the second function. DFA showed overall efficiency of 90.9% for the discrimination of groups. DFA performed on the head measurements dataset gave two canonical discriminant functions in which the first is significant ($P < 0.005$) and the second is close to significant ($P = 0.052$). On the scatterplot specimens are divided in three group (fig. 3), with sleight overlap on both discriminant functions. DFA showed overall efficiency of 71.1% for the discrimination of groups.

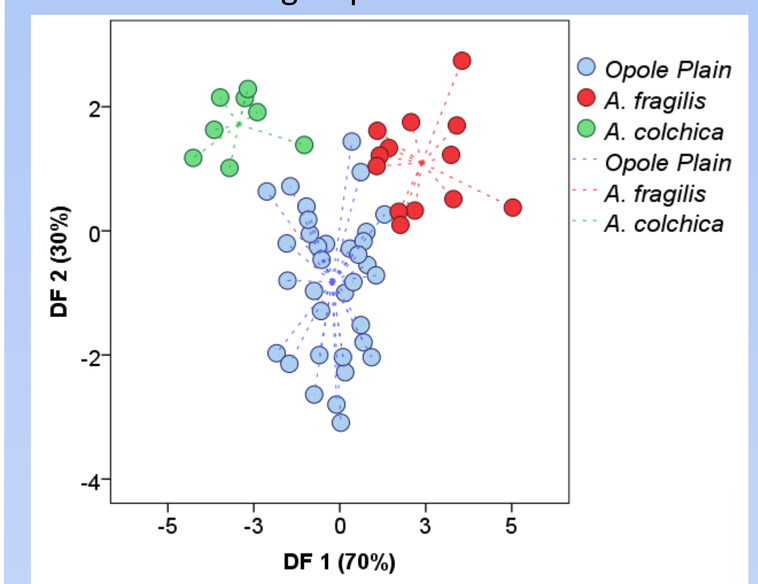

Fig. 2. Values of canonical discriminant functions for slow worms (truss network + meristic characters) of three groups. Only the first function is significant ($P < 0.001$). Spikes are crossing in centroids.

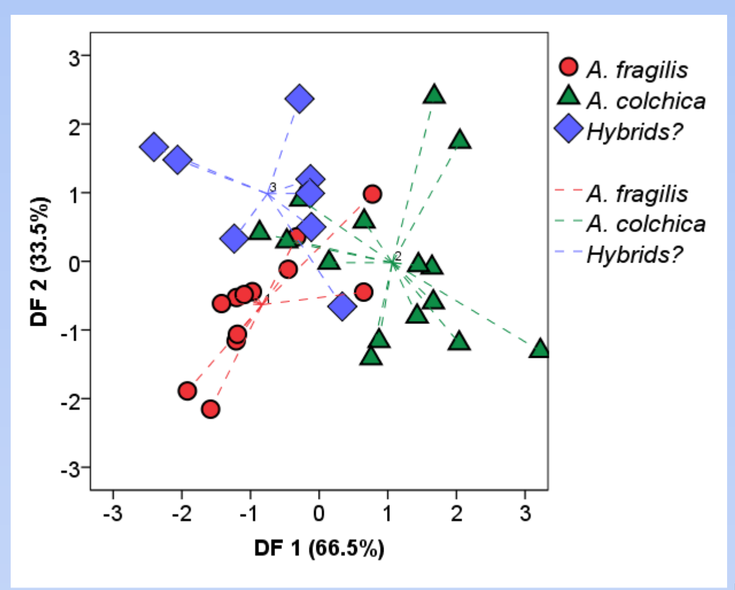

Fig . 3. Values of canonical discriminant functions for slow worms ("traditional" morphometry) of three groups. Spikes are crossing in centroids.

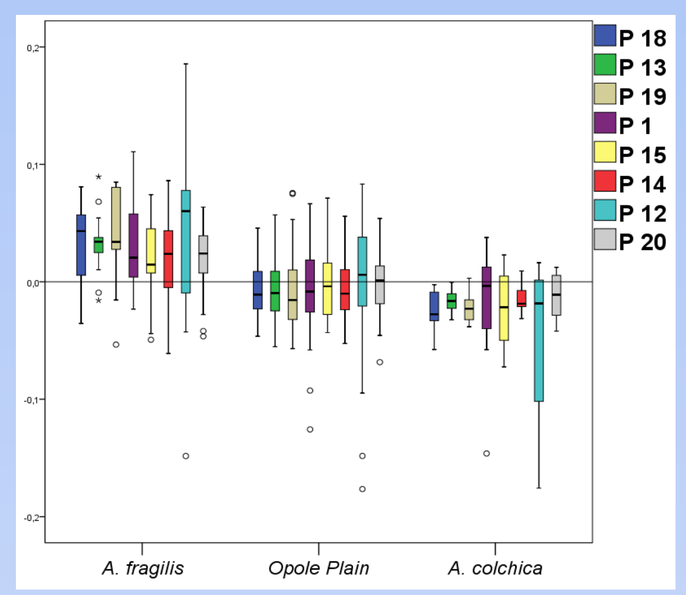

Fig. 4. Statistics for measurements of truss network for three populations. Presented distances are the most correlate with the first canonical discriminant function and significantly discriminate groups. Horizontal line inside the box = median, upper box value = 75th percentile, lower box value = 25th percentile, whiskers = maximum or minimum value, dot = outlier value, asterix = extreme value.

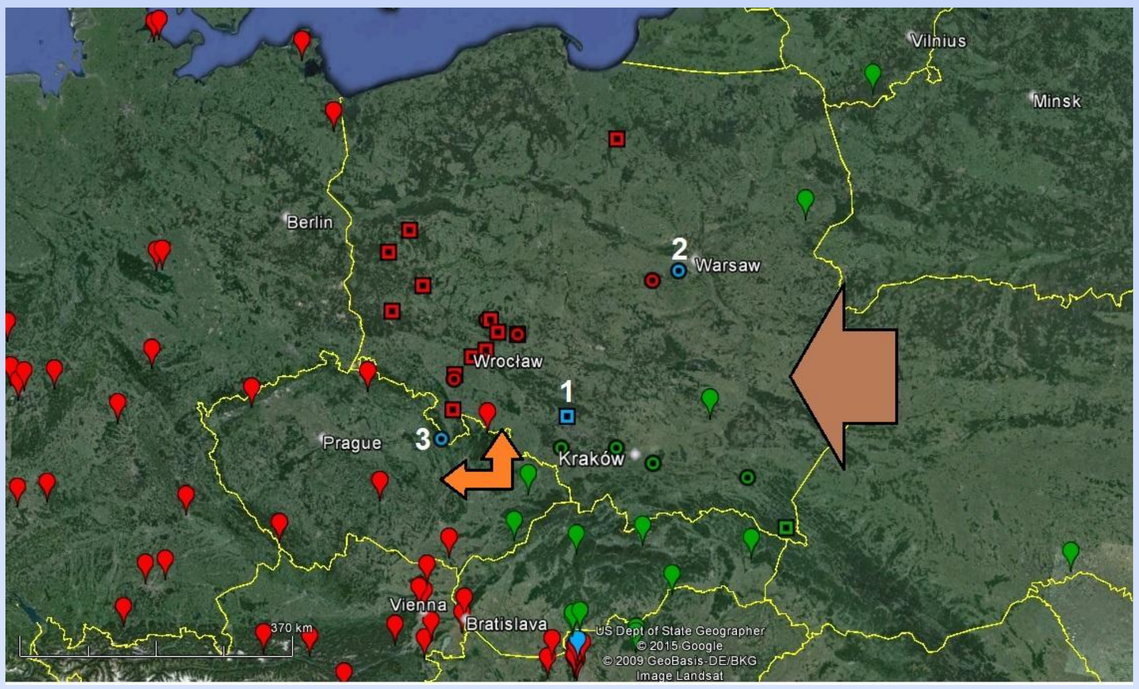

Fig. 5. Distribution of *Anguis* species in Poland. Red = *A. fragilis*, green = *A. colchica*, blue = hybrid/introgression (1 = eastern part of Opole Plain, 2 = Podkowa leśna, 3 = Niemojów), squares = truss network + meristic characters, circles= "traditional" morphometry, paddle = Gvoždík et al., 2013 or Szabó and Vörös, 2014, single arrow = probably main direction of *A. colchica* colonization, branched = probably minor direction of *A. colchica* colonization. Image generated from Google Earth.

The intermediate morphotype of Opole Plain, Podkowa Leśna and Niemojów specimens may be explained by the hybridization or existence of introgression between *A. fragilis* and *A. colchica* in the territory of Poland. The similar situations are well known in reptiles and other animals (Aird at al., 1989; Arnold, 1999). The eastern part of Poland was probably colonized by slow worms from the eastern part of Europe (Ukraine, Belarus, Lithuania). We found also specimens of *A. colchica* in the southern Poland (near Rybnik and Kraków). This suggest that the second colonization route is the Moravian Gate. Additionally results of truss network analysis (specimens from Opole Plain) support this statement (fig. 5). This could be confirmed by molecular research. Specimens from Rybnik and Podkowa Leśna suggest that Wisła river is not border for this species (or it is a border from two population of *A. colchica*, first from East Europe, second from Moravian Gate).

4. References

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