Climate reconstructions based on Miocene leaf flora from NW Bulgaria: Comparing leaf physiognomy and nearest living relative approach

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Abstract. The problem of paleoclimate reconstruction is an extremely interesting issue, which has been repeatedly discussed in many publications. This topic engages the attention of numerous specialists in many and various scientific disciplines. Fossil plants have vast potential as a source of information about past climatic conditions in the terrestrial realm. Various methods have been developed for the extraction of climate information from fossil land plants, but only few of these methods have provided quantitative data, e.g. Leaf Margin Analysis, Leaf Area Index, CLAMP and Coexistence Approach (CA). In this study we analyzed Middle Miocene floras from Bulgaria aiming to compare the results from different methods. The fossil floras are located in the southernmost part of the Forecarpathian Basin (NW Bulgaria). Two types of models were used to obtain quantitative data about the paleoclimate characteristics in the studied area – Simple Linear Regression (SLR) and Multiple Linear Regression (MLR) model. Furthermore, CLAMP and CA were applied. The obtained results evidence overlapping of CLAMP and CA data for diverse floras, while CLAMP data tend to produce cooler estimates than those obtained with the CA. The temperatures calculated by the SLR and MLR models are more or less consistent, but only when the standard deviations are considered. Moreover, the SLR and MLR intervals have strong correlation with those obtained from the CA. This corroborates statements by other authors, that under favorable circumstances (high diversity of the fossil flora and good taxonomic resolution) the climatic resolution of the CA can be twice as high compared to Leaf Physiognomy Approaches. The results obtained from the CA have less variability, consistently with data obtained from the MLR model. A great advantage of the CA method is that the width of coexistence intervals does not depend on species richness.

Key words: climate; Climate Leaf Analysis Multivariate Program (CLAMP); coexistence approach; leaf margin analysis; Miocene; Bulgaria
Introduction

The problem of paleoclimate reconstruction is an extremely interesting issue, which has been repeatedly discussed in many publications. This question engages the attention of a number of specialists in many and various scientific disciplines and many methods have been developed for its solving. The main problem is that the source of information should be properly chosen. Fossil plant remains have great potential as a source of information about past climatic conditions in the terrestrial realm. Plant adaptation to environmental conditions is more or less directly connected to morphological modifications in their organs. Thus, the morphological characteristics of the fossil plant remains can be used as indicators of paleoclimatic conditions.

Various methods have been developed for extraction of climate information from fossil land plants, but only few of these methods have provided quantitative data. Bailey & Sinnott (1915, 1916) were the first to observe that the percentage of woody dicotyledonous species with entire-margin leaves is higher in tropical floras than in cooler climatic zones. That gives a base for development of methods based on correlation between leaf physiognomy and climate parameters – Leaf Margin Analysis (Wolfe 1971, 1979), Leaf Area Index, and CLAMP (Wolfe 1993). The application of these methods is relatively simple because it does not require a detailed taxonomical determination. That's why the leaf physiognomy approach methods can be easily applied, also on floras having not any / no up-to-date taxonomical treatment.

Another widely used technique for palaeoclimate reconstruction is based on the assumption that the climatic requirements of fossil species are more or less similar to those of their nearest living relatives (NLRs). This technique is known as the “Nearest Living Relative” Method (Chaloner & Creber 1990). A recent variation of this method is the so-called Coexistence Approach, described by Mosbrugger & Utescher (1997).

Each of these methods has its advantages but they must be compared and analyzed, in order to find the most precise of them. Such comparisons of different methods (e.g. Uhl & al. 2003 on Oligocene and Middle Miocene floras from Germany) show that application of different methods for palaeoclimate reconstructions contributes to improving the basis for evaluation of climate parameters and reduces the influence of sources of errors.

Study area

Our study area is located in NW Bulgaria and represents the southernmost part of the Forecarpatian Basin. The Forecarpatian Basin represents the eastern part of the Central Paratethys (Fig. 1) and is a key region to understand the Neogene evolution of the connection between the Central and the Eastern Paratethys area (Rögl 1998, Meulencamp & Sissingh 2003). Apparently, the Forecarpatian Basin also played a major role in the evolution and migration of Mediterranean vegetation (Palamarev 1989).

At the beginning of the Middle Miocene, a large marine transgression flooded most of
Northwest Bulgaria, which became part of the Forecarpathian Basin (Fig. 2). Before the end of the Badenian, the sea retreated from the territory of NW Bulgaria, and a new transgression occurred in the Volhynian. The longitudinal depression and the marginal stable area in the south were covered by seawater.

The extension of the Volhynian Basin was approximately the same as in the Lower Badenian. During the Bessarabian, subsidence of the longitudinal depression ceased and the area was nearly filled up with sediments. Sedimentation stopped during the Chersonian. The fossil floras of this area have been studied on the base of leaf imprints, seeds/fruits and dispersed cuticles (Hadjiev & Palamarev 1962; Palamarev & Uzunova 1970, 1992; Palamarev & al. 1975, 1978; Petkova 1967; Petkova & Kitanov 1965; Palamarev 1988, 1990, 1993; Palamarev & Petkova 1987; Uzunova 1995, 1996). In the present study we have re-analyzed these floristic data with the help of the Bivariate (SLR) and Multivariate (MLR and CLAMP) analyses in order to obtain quantitative data about the Volhynian and Bessarabian climate evolution in the Southern Forecarpathian Basin. Ivanov & al. (2002) gave palynological evidences for Middle and Upper Miocene climate changes in the Forecarpatian Basin using the Coexistence Approach method. They estimated that during the Volhynian and the greater part of the Bessarabian Mean Annual Temperature (MAT) was between 15.6–17.2°C, Cold Month Mean Temperature (CMMT) mainly between 5–7°C and summer temperatures (WMMT) within 24.6–27.8°C.

The analyzed floristic assemblages originate from brackish sediments of the Forecarpathian Basin, which are Volhynian and Bessarabian in age as dated by foraminifera, mollusks and ostracods (Kojumdjieva & al. 1989). The source of taxonomic data for our study are palaeofloristic studies of Petkova (1967), Petkova and Kitanov (1965), Palamarev (1988, 1990, 1993), and Palamarev & Petkova (1987).

We analyzed four floras of Volhynian age located near the villages of Ruzhintsi, Tsar Shishmanovo, Cladorub-Ostrokaptsi and Pelovo, and one flora of Bessarabian age located between the villages of Belo Pole and Cherno pole. (Table 1).

The present-day climate of Northwest Bulgaria is characterised by MAT 11.2–11.5°C, CMMT −2.1 to −0.9°C, WMMT 22.6 to 23.6°C, and Mean Annual Precipitation (MAP) 536 to 586 mm (Velev, 1997).

Fig. 2. Sketch map showing the structural / palaeogeographical areas in Northwest Bulgaria during the Neogene and location of analyzed floras.

Analyses

Leaf physiognomy approach

Climate reconstruction methods connected with leaf physiognomy are based on the mathematical correlations between the morphological characters of the leaves (such as leaf margin type, leaf shape, venation type, etc.) and some climatic parameters (such as mean annual temperature, growing season precipitation, etc.). These correlations can be expressed as linear regression equations and they can be used for estimation of the values of some climatic parameters. In order to find the most statistically reliable equations, the pairs (leaf character – climatic parameter) with the strongest correlations should be found. As a criterion, the so-called coefficient of correlation was used which measures linear relation, if any, between the values of two parameters.

Two types of models were used to obtain quantitative data about the paleoclimate characters in the studied area: Simple Linear Regression (SLR) and Multiple Linear Regression (MLR) models. The SLR model uses only one predictor variable (one leaf character) for estimation of the response (climatic parameter). These relations could be illustrated by plots, or expressed as simple linear regression (SLR) equations:

\[
\text{MAT} = 0.267 \times (\%\text{No Teeth}) + 1.747 \quad r^2 = 0.932
\]

\[
\text{WMMT} = 0.170 \times (\%\text{No Teeth}) + 15.190 \quad r^2 = 0.875
\]

\[
\text{CMMT} = 0.305 \times (\%\text{No Teeth}) - 11.081 \quad r^2 = 0.947
\]

In order to calculate the SLR models, we used the percentages of leaves that have entire margins (\%No Teeth) Wolfe’s CLAMP 3B database as a predictor variable for MAT, WMMT, and CMMT. These three climate characters have the strongest correlations with “\%No Teeth” (\%Entire) (percentage of woody dicotyledonous species with entire margin leaves) leaf character.

MLR models use more than one predictors for estimating the response. These relations are expressed as multiple linear regression (MLR) equations. In order to find the best MLR equations for calculating MAT, WMMT and CMMT we experimented with a number of combinations of leaf characters and arrived at the following equations:

\[
\text{MAT} = 0.197 \times (\%\text{No Teeth}) - 0.180 \times (\%\text{Leptophyll 2}) + 0.080 \times (\%\text{Emarginate Apex}) + 0.011 \times (\%\text{Acute Base}) - 0.185 \times (\% <1:1) + 0.029 \times (\%1–2:1) - 0.002 \times (\%\text{Obovate}) + 5.693 \quad r^2 = 0.947
\]

\[
\text{WMMT} = 0.138 \times (\%\text{No Teeth}) - 0.056 \times (\%\text{Leptophyll 2}) - 0.013 \times (\%\text{Emarginate Apex}) + 0.007 \times (\%\text{Acute Base}) - 0.233 \times (\% <1:1) + 19.387 \quad r^2 = 0.818
\]

\[
\text{CMMT} = 0.222 \times (\%\text{No Teeth}) - 0.210 \times (\%\text{Leptophyll 2}) + 0.233 \times (\%\text{Emarginate Apex}) + 0.057 \times (\%\text{Acute Base}) - 0.111 \times (\% <1:1) - 0.022 \times (\%1–2:1) - 0.010 \times (\%\text{Obovate}) - 4.216 \quad r^2 = 0.970
\]

CLAMP (Climate–Leaf Analysis Multivariate Program) is another climate reconstruction model that uses the Canonical Correspondence Analysis (CCA). CCA integrates the regression and ordination methods, i.e. it relates the species abundances to explanatory variables, with no assumption of linearity in the data. In CCA, ordination axes, which are linear combinations of the explanatory variables, are extracted so as to maximize the dispersion among the species, with the constraint that the ordination axes must be uncorrelated with each other (i.e. they are orthogonal) (Wiemann & al. 1998).

The regression coefficients of SLR, MLR equations and the CLAMP model were computed with data from Wolfe’s CLAMP 3B database, which is a tabulation of data on 31 leaf characters and 11 meteorological parameters from 173 sites located in temperate and tropical America and Japan. The regressions were estimated with SPSS and the CLAMP model was calculated with the help of CANOCO for Windows software.

Table 1. Investigated floras with geographical location, stratigraphy, sample size and diversity.

<table>
<thead>
<tr>
<th>Location</th>
<th>Latitude</th>
<th>Longitude</th>
<th>Altitude</th>
<th>Stratigraphic level</th>
<th>Number of specimens</th>
<th>Number of taxa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kladorub – Ostrokaptsi</td>
<td>43° 43’ E</td>
<td>22° 39’ N</td>
<td>193 m</td>
<td>Volhynian</td>
<td>44</td>
<td>19</td>
</tr>
<tr>
<td>Ruzhintsi</td>
<td>43° 37’ E</td>
<td>22° 50’ N</td>
<td>181 m</td>
<td>Volhynian</td>
<td>587</td>
<td>82</td>
</tr>
<tr>
<td>Tsar Shishmanovo – Tolovitsa</td>
<td>43° 46’ E</td>
<td>22° 34’ N</td>
<td>229 m</td>
<td>Volhynian</td>
<td>63</td>
<td>15</td>
</tr>
<tr>
<td>Pelovo</td>
<td>43° 27’ E</td>
<td>24° 16’ N</td>
<td>110 m</td>
<td>Volhynian</td>
<td>73</td>
<td>27</td>
</tr>
<tr>
<td>Belo pole – Cherno pole</td>
<td>43° 37’ E</td>
<td>22° 54’ N</td>
<td>234 m</td>
<td>Bessarabian</td>
<td>18</td>
<td>11</td>
</tr>
</tbody>
</table>
After calculating the regression models, all sites for which the difference between estimated and observed value exceeded 3°C (for MAT, WMMT and CMMT) were eliminated, assuming that they are outliers. Then the models were calculated again using the remaining sites.

**Nearest living approach**

Since the first attempt at using the nearest living approach in the 19-th century (Heer 1855, 1856, 1859), it became a widely applied technique for palaeoclimate estimates with the help of fossil plants. It is based on comparisons of fossil taxa with recent species and it is assumed that the climatic requirements of fossil species are more or less similar to those of their nearest living relatives. In our case we applied the so-called coexistence approach method (Mosbrugger & Utescher, 1997) to obtain quantitative climatic data. This technique is straightforward and requires two steps. First, for all taxa of a given fossil flora the nearest living relatives and their climatic tolerances with respect to various climatic parameters are determined; then the interval is calculated for the various climate parameters, within which all nearest living relatives of the fossil flora can coexist. This coexistence interval is regarded as representing a reasonable estimator of the past climate under which the fossil flora lived. Such approach can be used with all kinds of plant remains (e.g. leaves, fruits/seeds, pollen/spores) for which the NLRs can be reliably identified. The method was recently applied for paleoclimate reconstructions in Europe and Asia (e.g. Pross & al. 1998; Utescher & al. 2000, Ivanov & al. 2002, 2011, Ivanov & Worobiec 2017, Ivanov & Lazarova 2019). For a given fossil flora, the CA method determines the nearest living relatives of fossil taxa and their climatic tolerances and calculates the coexistence intervals (minimum and maximum values) for various climate parameters (for details see Mosbrugger & Utescher 1997 and Utescher & al. 2014) within which all living relatives of fossil species can coexist.

**Results and discussion**

Fig. 3 shows the results of the application of the regression equations, CLAMP model and the result obtained from Coexistence Approach. The results obtained for the Volhynian show similar values, slightly lower intervals concerning Kladorub-Ostrokaptsi, which could be due to the incompleteness of the flora and the lower number of species with entire-margined leaves, which is the main temperature signal in

![Fig. 3. Paleoclimate data for the studied floras based on the application of the regression equations, CLAMP model and Coexistence Approach.](image)

Abbreviations: SLR – Simple Linear Regression model; MLR – Multiple Linear Regression model; CLAMP – Climate-Leaf Analysis Multivariate Program, and CA – Coexistence Approach.
the multivariate data set. Uhl & al. (2003) indicated the phenomenon that entire-margined leaf taxa are underrepresented in the specimen-poor floras. Such accumulation of toothed leaves could be due to the mixing of two different floral elements that originated from different places. That is why the zonal and azonal elements must be carefully determined. Kladorub-Ostrokaptsi flora has the lowest specimen number as compared to the rest of the Volhynian localities, which could possibly explain the lower temperature intervals calculated for this locality. The rest of the Volhynian localities show consistent temperature intervals, which is an evidence of relatively stable climatic conditions during that period. Moreover, these intervals have a strong correlation with the intervals obtained from the palynological investigations (Ivanov & al. 2002). The data for Bessarabian shows slightly lower values of reconstructed temperature parameters that might represent climate changes, but also could be explained by low taxonomic diversity and incompleteness of the fossil record. The temperature intervals obtained for the Bessarabian are wider, owing to the higher values of standard deviation as a result of the lower number of species identified in that flora.

This study provides interesting results regarding the precision of the different approaches. Generally, the temperatures calculated by SLR and MLR models are more or less consistent, but only when the standard deviations are considered. Moreover, the SLR and MLR intervals have a strong correlation with those obtained from Coexistence Approach. Even when including the standard deviation, the CLAMP intervals tend to be lower and stay slightly out of the main tendency. However, CLAMP and CA results overlap in 86.7% of cases (cf. fig. 3), a very high level compared to findings of Uhl & al. (2003; 2007) comparing CA and CLAMP data obtained from Central European Miocene floras, Wilf (1997) expressed an opinion that the large number of predictor variables, most of which without correlation with the temperature parameters used in the CLAMP analysis, may affect the results. At first sight, the same problem may arise at application of the MLR technique; however, a careful selection of predictor variables can only increase the precision of calculations.

The standard deviations of the three methods based on Leaf Physiognomy Approach are almost equal, but in any case, they are much greater than the interval ranges produced by the Coexistence Approach. The standard deviations are strongly dependent on the number of taxa in a given flora, thus the results obtained from species-poor floras should be interpreted carefully. This account corroborates the statements of other authors (Uhl & al. 2003) that the climatic resolution of Coexistence Approach is twice higher than the one obtained from the Leaf Physiognomy Approaches (LPhA). Above all, the results obtained from the Coexistence Approach show much lower variation intervals, which are more or less relevant to those obtained from the LPhA. A great advantage of the CA method is that the width of coexistence intervals does not depend on the species richness. Irrespective of the used method, mention deserves the fact that the results represent climate parameters of a small spot, whose values could be influenced by some local geographical features.

**Conclusions**

Confidence intervals obtained from the Leaf Physiognomy Approach (LPhA) for all studied floras are greater than the ranges obtained from the Coexistence Approach (CA) and are directly connected to the number of taxa in the flora. The dataset for LPhA includes data from small spots, which could strongly depend on microclimate. Moreover, it contains information only about sites located in temperate and tropical America and Japan, but not in Europe. The application of Leaf Physiognomy Approach probably requires more detailed vegetation analyses, in order to eliminate the influence of azonal and intrazonal vegetation elements. The CLAMP intervals overlap in the vast majority of cases with the CA ranges, but tend to be lower, especially for less diverse floras, and stay slightly out of the main tendency. This probably is due to the large number of predictor variables, most of which without correlation with the temperature parameters used in CLAMP analysis, and this can disturb the results. At first sight, the same problem should arise during application of the MLR technique. However, this could be avoided, if the predictor variables are carefully selected. Only then they can increase the precision of the results.

The precision of the CA method strongly depends on the proper determination of fossil taxa and proper assignment of the nearest living relatives (NLRs). The climatic tolerance of the NLRs may differ from
the climatic tolerance of the corresponding fossil taxa, and that should be taken under consideration, especially in the case of species with a much wider area in the past than at present. Recent updates of the Paleoflora Database (Utescher & Mosbrugger 2015) have significantly improved the precision of methods using both macro- and micro-palaeobotanical data.

The data obtained in the present study confirm that the Volhynian in Northwest Bulgaria was a period with warm temperate climate with annual temperatures between 14.2–17.1°C, verified also by the pollen data provided by Ivanov & al. (2002). For the Bessarabian a trend to slightly lower temperature is observed that could be interpreted as the beginning of a climatic change.

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References:


Ivanov, D., Worobiec, E. 2017. Middle Miocene (Badenian) vegetation and climate dynamics in Bulgaria and Poland based on pollen data. – Palaeogeogr. Palaeoclimatol. Palaeoecol., 467, 83-94.


Palamarev, E. 1990. Fundamentals of the palaeofloristic palaeosuc- cession in the Late Miocene (Sarmatian Pontian) in Bulgaria. – In: Palaeofloristic and palaeoclimatic changes in the Cretaceous and Tertiary, Prague, 257-263.


