

Evaluating the paleoenvironmental significance of tricyclic terpanes: Evidence from Miocene fine-grained sediments of the Danube Basin (Slovakia)

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Abstract: This study investigates the distribution of tricyclic (C_{19} – C_{26}) and tetracyclic terpanes in thirty fine-grained sediment samples from the Kolárovo-4, Pozba-4, and Zelený háj-1 wells to reconstruct paleoenvironmental conditions in the Miocene Danube Basin in Slovakia (NW Pannonian Basin System). The results reveal clear spatial and stratigraphic variations reflecting the transition from the Sarmatian to the Pannonian stages. In the Middle Miocene (Sarmatian) Vráble Formation, a predominance of C_{23} TT is consistent with marine to saline lacustrine conditions, followed by a shift to fluvial–deltaic and freshwater lacustrine environments with higher C_{19} and C_{20} homologs. Within the Upper Miocene Pannonian, the offshore Ivanka Formation shows mixed tricyclic terpane patterns that suggest a transition from marine–brackish to freshwater lacustrine settings. The overlying Upper Miocene Beladice Formation is dominated by C_{21} TT and C_{23} TT, consistent with stable freshwater-influenced delta–plain sedimentation. Overall, tricyclic terpanes represent useful complementary geochemical indicators that, when interpreted together with stratigraphic and sedimentological information, may aid the reconstruction of basin evolution and depositional environments across major paleoenvironmental changes in the Miocene Danube Basin.

Keywords: Tricyclic terpanes, Paleoenvironmental proxies, Beladice Fm., Ivanka Fm., Vráble Fm., Depositional environment

1. INTRODUCTION

The Miocene evolution of the Danube Basin was characterized by major paleoenvironmental changes associated with the transition from the marine Central Paratethys to the lacustrine Lake Pannon system (e.g. Kováč et al., 2018^a; Vlček et al., 2024). Reconstructing these environmental changes requires the integration of multiple geological and geochemical approaches, including sedimentology, stratigraphy, paleontology, and organic geochemistry. Organic geochemical data are particularly valuable because sedimentary organic matter retains molecular information related to depositional conditions, organic matter sources, and post-depositional processes. Consequently, the distribution of specific biomarker compounds has been widely applied in paleoenvironmental studies to evaluate variations in depositional settings, redox conditions, and organic matter input (Peters et al., 2005).

Among the various biomarker groups, tricyclic terpanes (TT) have attracted considerable attention because of their widespread occurrence in sediments and petroleum systems and their relative resistance to biodegradation. Numerous studies have demonstrated their usefulness in source-rock characterization, oil-source correlations, thermal maturity assessment, and paleoenvironmental reconstruction (Grosjean et al., 2012; Xiao et al., 2019). Although several biological precursors have been proposed, the exact origin of tricyclic terpanes remains debated. Polyprenols derived from bacterial and archaeal membranes are commonly regarded as important precursors, while higher plants have also been suggested as potential contributors (Aquino Neto et al., 1982; Ourisson et al., 1982; Peters & Moldowan, 1993; Simoneit et al., 1993; Xiao et al., 2024).

Although tricyclic terpanes are widely used as paleoenvironmental proxies, their distributions may be influenced not only by depositional environment but also by source facies, lithology, thermal maturity, biodegradation, and variations in organic matter input. In addition, differences between carbonate and siliciclastic depositional systems may affect biomarker distributions. Therefore, tricyclic terpane parameters should be interpreted together with independent geological and geochemical evidence to improve the reliability of paleoenvironmental reconstructions (Peters et al., 2005; Xiao et al., 2024).

The aim of this study is to determine the applicability of tricyclic terpanes in the Miocene fine-grained sediments of the NW Pannonian Basin System, deposited in various environments including marine, lacustrine and deltaic. The results will then be compared with existing sedimentological and paleontological data. Additionally, the study investigates whether a change in composition occurs at the transition between the Sarmatian and Pannonian age, which represents a major paleoenvironmental turning point of the region (e.g., Kováč et al., 2018^a; Vlček et al., 2024).

2. GEOLOGICAL SETTINGS

The Danube Basin represents the northwestern sub-basin of the Pannonian Basin System (Fig. 1). The basin was opened in back-arc setting during the Middle Miocene as a response to the extension induced by slab rollback of the subducting Carpathian lithosphere, escape tectonics of the ALCAPA microplate after the collision with Europe, and thermal weakening and

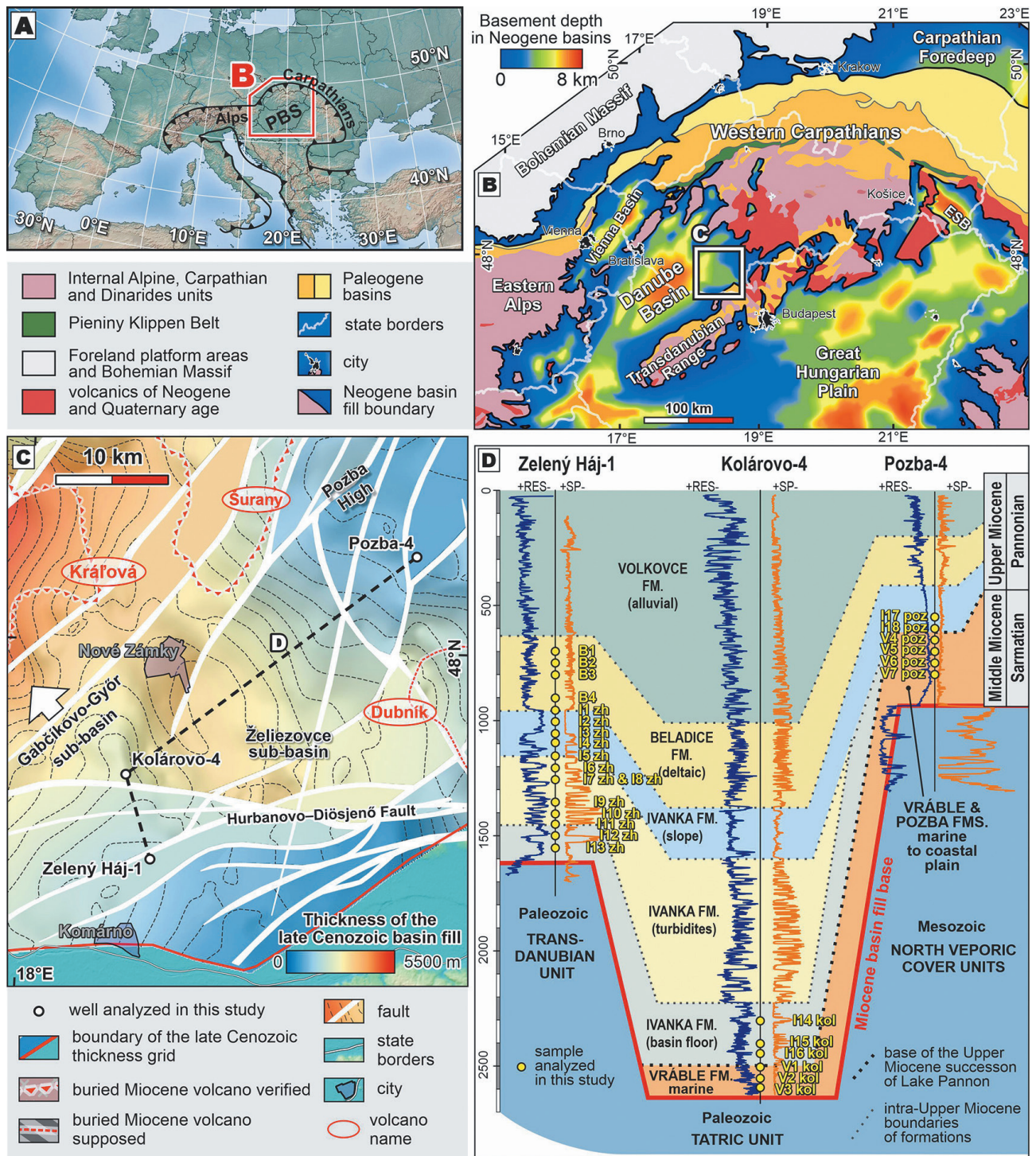


Fig. 1. Location of the study area. **A:** The Pannonian Basin System (PBS) between the arc of the Carpathians, Eastern Alps and Dinarides. **B:** Location of the study area in the eastern part of the Danube Basin. **C:** Map of the thickness of Cenozoic basin fill according to Šujan et al. (2025) with location of the studied wells. **D:** Stratigraphic correlation between the studied wells according to Vlček et al. (2022, 2024) with position of the analyzed samples. RES - resistivity well log, SP - spontaneous potential well log

asthenospheric upwelling (Kováč, 2000; Horváth et al., 2015; Klučiar et al., 2016; Kováč et al., 2017; Šujan et al., 2021, 2025; Csontos et al., 2025). The polyphase rifting of the basin began around 15 Ma (Kováč et al., 2018^a, 2018^b; Sant et al., 2017; Sant et al., 2020; Šujan et al., 2021), with the first phase recorded by the volcano-sedimentary deposits of the Bajtava-Špačince

formations (Vass, 2002; Šujan et al., 2021, 2025), which are of the lower Badenian age (Langhian; sensu Kováč et al., 2018^a, 2018^b). The next two syn-rift phases occurred during the late Badenian and Sarmatian (Serravallian) with the deposition of the Báhoň-Pozba and Ripňany-Vrable formations, respectively. In the depocenters, sandy deltaic, muddy inner shelf and shelf

break-slope deposits prevailed in this time (Fordinál & Elečko, 2000; Kováč et al., 2006, 2008, 2018^a, 2018^b; Rybár et al., 2015, 2016; Šarinová et al., 2018; Hudáčková et al., 2020; Vlček et al., 2020^a). The Vráble Formation analyzed in this study was accumulated in marine offshore to coastal plain setting (Vlček et al., 2022; Šujan et al., 2025).

During the Late Miocene, the marine gateways gradually ceased due to the uplift of the Carpathians (Vasiliev et al., 2010; ter Borgh et al., 2013), and the area was flooded by the brackish waters of Lake Pannon (Vass 2002; Magyar et al., 2013; Sztanó et al., 2016; Šujan et al., 2016, 2021; Magyar, 2021). The lacustrine Pannonian deposits are included in the Ivanka Formation, which comprises from bottom to top (1) muddy sediments deposited mostly by suspension settling in an offshore open lake environment, (2) dominantly sandy turbidite deposits, overlain by (3) muddy shelf-break slope strata (Sztanó et al., 2016). These sediments are replaced by deltaic strata of the Beladice Formation, which was accumulated on topsets of shelf slope clinoforms of the uppermost Ivanka Formation and mirrors normal regression of Lake Pannon (Sztanó et al., 2025). The Pannonian cycle culminates in alluvial sediments of the Volkovce Formation with variable composition, dominated with overbank deposits in more central areas with higher accommodation rates, while peripheral areas show higher proportion of sandy and even gravelly channel fills (Šujan et al., 2017, 2020, 2021; Joniak et al., 2020). The last depositional cycle takes place in the latest Late Miocene, Pliocene and Quaternary, and includes syn-basin inversion alluvial successions preserved mostly in the central depocentre (e.g., Vass, 2002; Kronome et al., 2012; Sztanó et al., 2016; Šujan et al., 2018, 2021).

3. SAMPLES AND METHODS

The sampling strategy was designed to capture major paleoenvironmental turnovers associated with the transition from the marine Paratethys to the lacustrine Lake Pannon and its subsequent regression during the Late Miocene. Particular emphasis was placed on fine-grained intervals spanning the Sarmatian–Pannonian boundary, which represents a fundamental shift from marine to brackish and freshwater depositional conditions in the Danube Basin. This transition provides a suitable natural framework for testing the sensitivity of tricyclic and tetracyclic terpanes as paleoenvironmental proxies.

A total of thirty mudstone samples were collected from three boreholes (Kolárovo-4, Pozba-4, and Zelený háj-1), covering offshore marine to lacustrine and deltaic depositional settings. The dataset includes samples from the Vráble Formation (Sarmatian), the Ivanka Formation (Pannonian offshore lacustrine), and the Beladice Formation (Pannonian deltaic), allowing evaluation of biomarker responses across contrasting salinity, redox, and organic matter input regimes associated with lake isolation and regression. The number of samples analyzed from each formation is summarized in Table 1, together with their depths and well affiliations.

Biomarkers were extracted from powdered rock samples using dichloromethane (DCM) and methanol (93:7, v/v) in a Dionex

ASE 100 system. Saturated and aromatic hydrocarbon fractions (SAT and ARO) were separated by silica gel column chromatography using n-hexane and DCM as eluents. The saturated hydrocarbon fraction was analyzed using an Agilent 6890N gas chromatograph coupled to an Agilent 5973N quadrupole mass selective detector (GC–MS). Chromatographic separation was performed on a DB-1MS capillary column (60.0 m × 0.250 mm i.d., 0.25 µm film thickness) using helium as the carrier gas at a constant flow rate of 1.3 mL/min. Sample injection (1 µL) was performed using an autosampler and a multimode inlet (MMI) operated in pulsed splitless mode. The MMI temperature program started at 60 °C (0.1 min), followed by heating at 900 °C/min to 270 °C and held for 3 min. The GC oven temperature program was 50 °C (2 min), increased at 2 °C/min to 310 °C and held for 20 min. Mass spectrometric detection was performed using electron ionization (EI) at 70 eV. The transfer line, ion source, and quadrupole temperatures were maintained at 280 °C, 300 °C, and 180 °C, respectively. Data acquisition was performed in SIM mode monitoring the characteristic ion m/z 191. Terpene distributions were evaluated from the m/z 191 mass chromatograms using Agilent MassHunter software.

The studied samples have been previously characterized using a broad range of organic geochemical parameters, including biomarker distributions, source-rock properties, and paleoenvironmental proxies (Vlček et al., 2020b; Vlček et al., 2022; Vlček et al., 2024). Previous studies also documented the presence of well-preserved n-alkanes in the aliphatic fractions and did not indicate evidence of significant biodegradation (Vlček et al., 2020b; Vlček et al., 2022; Vlček et al., 2024). Therefore, biodegradation is unlikely to represent the primary control on the tricyclic terpene distributions observed in the present study.

4. RESULTS

In the aliphatic fraction, several tricyclic terpanes were detected, such as C₁₉TT, C₂₀TT, C₂₁TT, C₂₂TT, C₂₃TT, C₂₄TT, and C₂₆TT, along with C₂₄TeT and C₂₆TeT (tetracyclic terpanes). Representative chromatograms for the m/z 191 ion are presented in figure 2, and the geochemical parameters derived from the abundance of specific tricyclic and tetracyclic terpanes are compiled in Table 1.

4.1 Vráble Formation (Sarmatian)

Samples from the Vráble Formation show moderate variability in tricyclic terpene ratios. The C₂₁/C₂₀TT ratios range from 0.76 to 1.16, while C₂₁/C₂₃TT ratios vary between 0.59 and 1.14. Ratios of short-chain homologs relative to C₂₃TT show C₂₀/C₂₃TT values between 0.51 and 1.38 and C₁₉/C₂₃TT ratios ranging from 0.18 to 0.54. Relative distributions indicate that C₁₉+C₂₀TT constitute 30.3–47.9%, C₂₁TT 25.6–29.6%, and C₂₃TT 25.0–43.9% of total tricyclic terpanes. The highest C₂₃TT contribution (43.9%) was recorded in the Kolárovo-4 (2510 m), whereas the lowest C₂₃TT value (25.0%) occurs in the Pozba-4 (800 m). This sample also shows the maximum proportion of short-chain homologs (C₁₉+C₂₀TT = 47.9%) within the formation.

Table 1. Organic geochemical parameters based on the abundance of tricyclic terpanes.

Sample	Well	Depth	Formations	C21/C20TT	C21/C23TT	C20/C23TT	C19/C23TT	C19+C20TT%	C21TT%	C23TT%
B1	Zh-1	700	Beladice Fm.	1.19	0.93	0.78	0.15	32.65	32.52	34.83
B2	Zh-1	749	Beladice Fm.	1.39	0.83	0.60	0.11	27.83	32.72	39.45
B3	Zh-1	801	Beladice Fm.	1.11	0.92	0.83	0.21	35.14	31.05	33.81
B4	Zh-1	904	Beladice Fm.	1.06	0.88	0.83	0.22	35.86	29.97	34.17
I1 zh	Zh-1	957	Ivanka Fm.	1.15	0.88	0.76	0.23	34.51	30.63	34.86
I2 zh	Zh-1	1004	Ivanka Fm.	1.01	1.13	1.11	0.34	40.61	31.46	27.93
I3 zh	Zh-1	1056	Ivanka Fm.	1.02	1.53	1.50	0.35	42.20	34.98	22.83
I4 zh	Zh-1	1095	Ivanka Fm.	0.84	1.72	2.05	0.67	50.08	31.55	18.38
I5 zh	Zh-1	1155	Ivanka Fm.	1.07	0.69	0.64	0.17	32.47	27.57	39.96
I6 zh	Zh-1	1205	Ivanka Fm.	1.25	0.70	0.56	0.17	30.04	28.83	41.13
I7 zh	Zh-1	1258	Ivanka Fm.	1.17	0.71	0.61	0.15	30.84	28.72	40.44
I8 zh	Zh-1	1259	Ivanka Fm.	0.64	0.96	1.51	0.14	45.69	26.55	27.76
I9 zh	Zh-1	1354	Ivanka Fm.	0.97	1.18	1.22	0.34	41.72	31.51	26.77
I10 zh	Zh-1	1407	Ivanka Fm.	1.04	0.59	0.56	0.12	29.95	25.87	44.19
I11 zh	Zh-1	1451	Ivanka Fm.	1.23	0.73	0.59	0.12	29.05	29.93	41.02
I12 zh	Zh-1	1503	Ivanka Fm.	1.24	0.42	0.34	0.08	22.81	23.00	54.19
I13 zh	Zh-1	1555	Ivanka Fm.	1.04	0.72	0.70	0.16	33.28	28.01	38.71
I14 kol	Kol-4	2303	Ivanka Fm.	0.71	0.65	0.92	0.45	45.31	21.54	33.15
I15 kol	Kol-4	2405	Ivanka Fm.	0.88	0.77	0.88	0.63	46.01	23.51	30.48
I16 kol	Kol-4	2450	Ivanka Fm.	0.44	0.57	1.28	1.07	60.01	14.53	25.46
I17 poz	Poz-4	550	Ivanka Fm.	0.98	0.91	0.93	0.32	39.61	28.74	31.65
I18 poz	Poz-4	600	Ivanka Fm.	0.89	1.03	1.16	0.32	42.06	29.37	28.57
V1 kol	Kol-4	2510	Vráble Fm.	1.16	0.59	0.51	0.18	30.25	25.89	43.86
V2 kol	Kol-4	2558	Vráble Fm.	1.10	0.60	0.55	0.20	31.84	25.61	42.56
V3 kol	Kol-4	2597	Vráble Fm.	0.96	0.69	0.72	0.27	36.95	25.67	37.39
V4 poz	Poz-4	650	Vráble Fm.	0.86	0.93	1.09	0.51	45.31	26.39	28.30
V5 poz	Poz-4	700	Vráble Fm.	0.96	1.14	1.19	0.53	44.53	29.56	25.92
V6 poz	Poz-4	750	Vráble Fm.	0.76	0.89	1.17	0.32	44.05	26.33	29.62
V7 poz	Poz-4	800	Vráble Fm.	0.79	1.09	1.38	0.54	47.87	27.16	24.98

Abbreviations: TT = tricyclic terpene. C19TT%, C21TT% and C23TT% represent the relative abundance (%) of individual tricyclic terpene homologues normalized to the sum of C19TT + C20TT, C21TT, C23TT.

4.2 Ivanka Formation (Pannonian)

The Ivanka Formation displays the widest range of tricyclic terpene distributions among the studied units. The C_{21}/C_{20} TT ratios range from 0.44 to 1.25, while C_{21}/C_{23} TT ratios vary broadly between 0.42 and 1.72. The C_{20}/C_{23} TT ratios span from 0.34 to 2.05, and C_{19}/C_{23} TT ratios range from 0.08 to 1.07. Relative abundances show substantial variability, with $C_{19}+C_{20}$ TT contributing 22.8–60.0%, C_{21} TT 14.5–35.0%, and C_{23} TT 18.4–54.2% of total tricyclic terpanes. The highest C_{23} TT proportion (54.2%) was identified in the Zelený háj-1 (1503 m), whereas the lowest C_{23} TT content (18.4%) occurs at Zelený háj-1 (1095 m). In contrast, the maximum contribution of short-chain homologs ($C_{19}+C_{20}$ TT = 60.0%) was observed in the Kolárovo-4 (2450 m).

4.3 Beladice Formation (Pannonian)

Samples from the Beladice Formation show relatively narrow and consistent ranges of tricyclic terpene ratios. The C_{21}/C_{20} TT ratios range from 1.06 to 1.39, and C_{21}/C_{23} TT ratios vary between 0.83 and 0.93. The C_{20}/C_{23} TT ratios range from 0.60 to 0.83,

while C_{19}/C_{23} TT ratios are low, between 0.11 and 0.22. Relative distributions indicate that $C_{19}+C_{20}$ TT account for 27.8–35.9%, C_{21} TT for 30.0–32.7%, and C_{23} TT for 33.8–39.5% of total tricyclic terpanes. The highest C_{23} TT contribution (39.5%) occurs in the Zelený háj-1 (749 m), while the lowest value (33.8%) was recorded at Zelený háj-1 (801 m). The maximum proportion of short-chain homologs (35.9%) is observed at Zelený háj-1 (904 m).

5. DISCUSSION

The interpretations presented below are linked primarily to depositional environments represented by the studied samples rather than to the formations as a whole, which may comprise multiple sedimentary facies and depositional settings.

5.1 Vráble Fm. – marine offshore to coastal plain (Sarmatian)

The Vráble Formation represents a heterogeneous depositional system within the Danube Basin, ranging from shallow marine

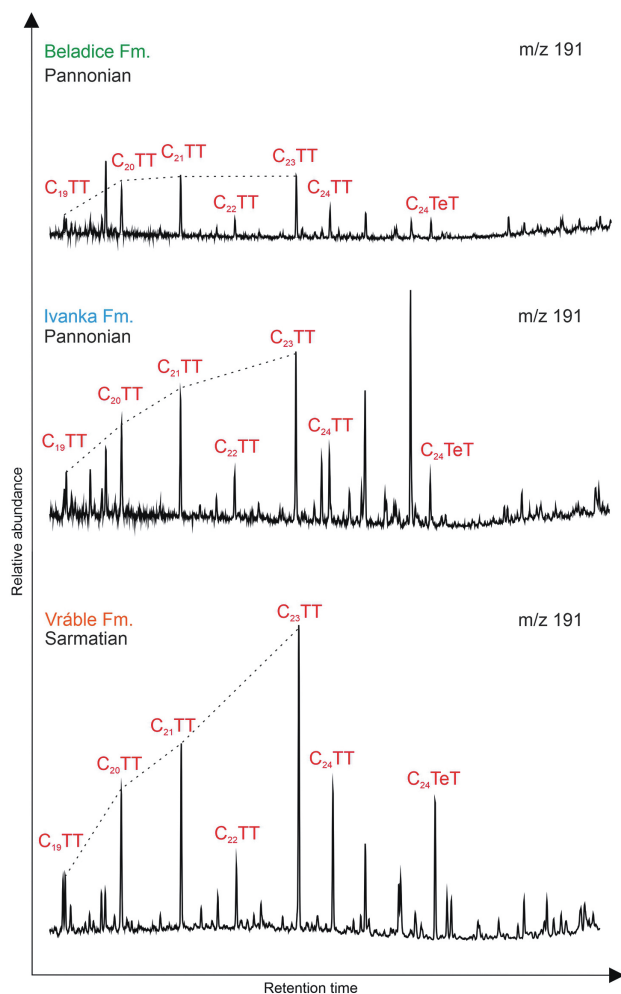


Fig. 2. Distribution of tricyclic terpanes (TT) and tetracyclic terpanes (TeT) in m/z 191 mass chromatograms. Samples represent deltaic to delta-plain facies (Beladice Fm.), offshore lacustrine facies (Ivanka Fm.), and offshore marine to marginal marine facies (Vráble Fm.).

to marginal and delta-influenced environments, as documented by sedimentological, biostratigraphic paleoenvironmental proxy studies (e.g., Kováč et al., 2018^a; Vlček et al., 2022; Vlček et al., 2024). Such environmental variability provides a suitable framework for evaluating the behavior of tricyclic terpanes across marine–coastal transitions.

The distribution patterns of tricyclic terpanes reveal systematic differences between the basin center and marginal settings. Samples from the Kolárovo-4 well are characterized by assemblages dominated by C_{23} TT, whereas samples from the Pozba-4 well show a relative enrichment of C_{19} TT and C_{20} TT (Fig. 3). This contrast may reflect a shift in organic matter sources and depositional conditions along a marine to marginal gradient.

These biomarker trends are consistent with a transition from more offshore-influenced environments toward settings affected by increased freshwater influence and terrestrial organic matter supply, in agreement with previous paleoenvironmental reconstructions of the Sarmatian in the Danube Basin (Vlček et al., 2022). Similar tricyclic terpane distributions have been reported from marginal and delta-influenced depositional

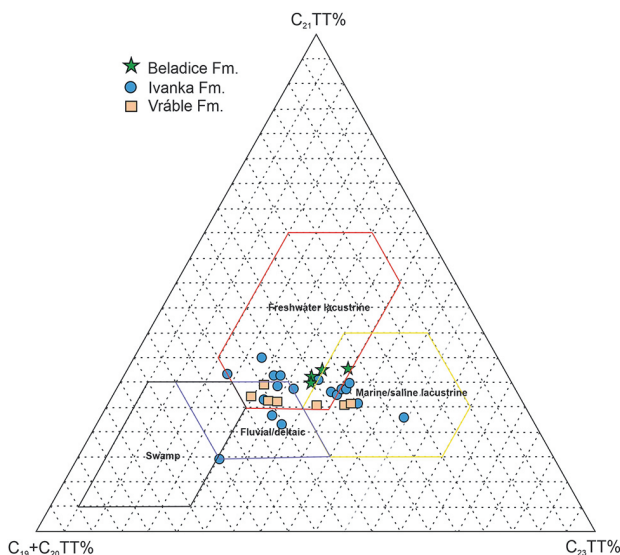


Fig. 3. Ternary diagram of $C_{19}+C_{20}$ TT, C_{21} TT, and C_{23} TT indicating variations in depositional settings. Depositional environment fields modified after Xiao et al. (2024).

settings in both marine and lacustrine systems, where short-chain homologues may become relatively enriched in association with enhanced terrigenous input and changing depositional conditions (Xiao et al., 2024; Jovanić et al., 2025).

5.2 Ivanka Fm. – offshore lacustrine (Pannonian)

The Ivanka Formation records the establishment and early evolution of Lake Pannon following the isolation of the Central Paratethys, representing a major paleoenvironmental turnover from marine to long-lived lacustrine conditions (Magyar et al., 2013; Sztanó et al., 2016). Previous studies documented pronounced spatial gradients in depositional conditions during this interval, related to basin isolation and lake evolution (Vlček et al., 2022; Vlček et al., 2024).

Tricyclic terpane assemblages show marked spatial variability across the studied wells, reflecting differences between more central and marginal parts of Lake Pannon. Assemblages dominated by C_{23} TT prevail in the western, more central parts of the basin, suggesting stronger aquatic/brackish influence, whereas short-chain homologs become increasingly important toward the eastern, marginal areas.

This systematic spatial pattern is interpreted to reflect increasing freshwater influence, enhanced terrestrial organic matter input, and increased restriction toward marginal sub-basins during the evolution of Lake Pannon (Fig. 3). This evolutionary scenario is specific to the study area, which experienced isolation from the main water body of Lake Pannon (Vlček et al., 2024), and hence its sensitivity to paleoenvironmental variations was significantly pronounced. Comparable tricyclic terpane distributions have been reported from other long-lived lake systems affected by lacustrine isolation and freshwater influence (Xiao et al., 2024). Similarly, elevated proportions of C_{23} TT are consistent with observations from brackish, saline lacustrine, and marginal-marine environments described by Jovanić et al. (2025).

5.3 Beladice Fm. – deltaic (Pannonian)

The Beladice Formation represents deltaic and delta-plain environments associated with the late-stage regression of Lake Pannon (Sztanó et al., 2016), as supported by sedimentological evidence and freshwater fossil assemblages (Vlček et al., 2024). Compared to the underlying Ivanka Formation, depositional conditions during Beladice time are considered to have been more stable and spatially uniform.

This interpretation is supported by comparatively uniform tricyclic terpene distributions across the studied interval, with limited spatial variability between samples. The relatively uniform TT assemblages suggest comparatively stable depositional conditions and a relatively balanced contribution of aquatic and terrestrial organic matter during delta progradation (Fig. 3). However, the position of the Beladice samples in the ternary diagram does not uniquely define freshwater delta-plain environments and is therefore interpreted as being consistent with freshwater-influenced deltaic conditions reconstructed independently from sedimentological and paleontological evidence.

Such stabilization of tricyclic terpene assemblages is consistent with shallow lacustrine to delta-plain environments developed during lake regression, where environmental gradients are less pronounced than during earlier stages of basin evolution. Similar patterns have been described from delta-influenced and shallow lacustrine successions, highlighting the resilience of tricyclic terpenes as paleoenvironmental indicators in siliciclastic systems (Xiao et al., 2024; Jovanić et al., 2025).

6. CONCLUSION

This study evaluates the paleoenvironmental significance of tricyclic (and tetracyclic) terpenes in Miocene siliciclastic successions of the Danube Basin spanning the transition from the Central Paratethys to Lake Pannon and its subsequent regression. By integrating biomarker distributions with stratigraphic and sedimentological data, the study assesses the applicability and limitations of tricyclic terpenes as paleoenvironmental proxies.

Tricyclic terpene assemblages show systematic variations between marine-influenced, lacustrine, and deltaic settings and broadly reflect changes in organic matter sources and depositional conditions. Elevated abundances of C_{23} TT are generally associated with marine-influenced and saline lacustrine environments, whereas increased proportions of C_{19} TT and C_{20} TT are consistent with enhanced terrestrial organic matter input. Elevated C_{21} TT abundances may be associated with freshwater-influenced conditions, consistent with previously published interpretations.

Overall, the results indicate that tricyclic terpenes represent useful complementary indicators of broad paleoenvironmental changes in siliciclastic basins. However, their distributions are influenced by multiple interacting factors, including depositional environment, organic matter sources, and early diagenetic processes, and should therefore be interpreted together with independent geological evidence.

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