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A contribution to the variability of *Prunus spinosa* L. in the vicinity of the mediaeval Castle Kolno, S-W Poland

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Abstract. The variability of fruit stone characteristics of medieval fossil forms and modern ones was analysed in a small *Prunus spinosa* (blackthorn) population in the vicinity of Castle Kolno near Stare Kolnie in Opole Province. A modern putative hybrid resulting from natural crossing with cultivated plums was discovered, corresponding to some fossil morphotypes. The analysis of the correlations of features indicated developmental relationships between them. The pattern of variability of the examined stones in the ordination space confirmed the development of the *Prunus spinosa*-*Prunus domestica* complex as an element of a dynamic syngameon. The population also showed variations in leaf morphotypes, which may be used in taxonomic analysis.

Key words: correlations, fruit stone, hybrid, morphometry, ordination analysis, *Prunus spinosa*

1. Introduction

Prunus spinosa (blackthorn) shrubs, together with other shrub species, form the ecotone communities of the *Rhamno-Prunetea* syntaxon at the junction of open habitats (meadows and arable fields) and wooded ones (Matuszkiewicz 2001; Popescu & Caudullo 2016). In the vicinity of the medieval Castle Kolno (near the village of Stare Kolnie in Opole Province), the existing components of this syntaxon are probably remnants of an earlier phase of vegetation on the outskirts of the fields and ruins of the castle. Earlier archaeobotanical research at the site showed the possibility of crossing *P. spinosa* with *P. domestica* and formation of a syngameon (Kosina & Marek 2021). A similar hybrid taxon derived from *P. spinosa* (*Prunus ×fruticans* Weihe) was described from Moravia, and the key identification features were provided (Kühn 1988). Hanelt (1997) describes *P. ×fruticans* Weihe as a pentaploid form derived from crossing the above-mentioned species and lists eight subspecies within *P. spinosa*. He also indicates the difficulty in differentiating the putative hybrid from *P. spinosa*. The eight subspecies of *P. spinosa* highlight this challenge. Fossil specimens of *Prunus* stones, including blackthorn, are abundant, which enables us to perform adequate morphometric analyses on such samples. Many medieval specimens from Douai in northern France date from 8th-11th centuries and represent the typical form of stones for the species, but also a different variety *macrocarpa*. It is assumed that this variety is hybrid in origin (van Zeist *et al.* 1994). Detailed morphometric analyses of blackthorn and cultivated plums from Denmark revealed that hybrids between them exist (Nielsen & Olrik 2001). An analysis of the variability of many traits, including stone traits, in contemporary blackthorn populations around Wrocław provides evidence for the frequent occurrence of hybrids, particularly in populations adjacent to domestic plum trees (Staszak 2004). The present study investigated blackthorn variability in a small population in the vicinity of the medieval Castle Kolno in both fossil and modern material.

2. Materials and methods

Part of the plant material analysed in this study was collected during an archaeological excavation in the former moat of Castle Kolno as well as from three jugs

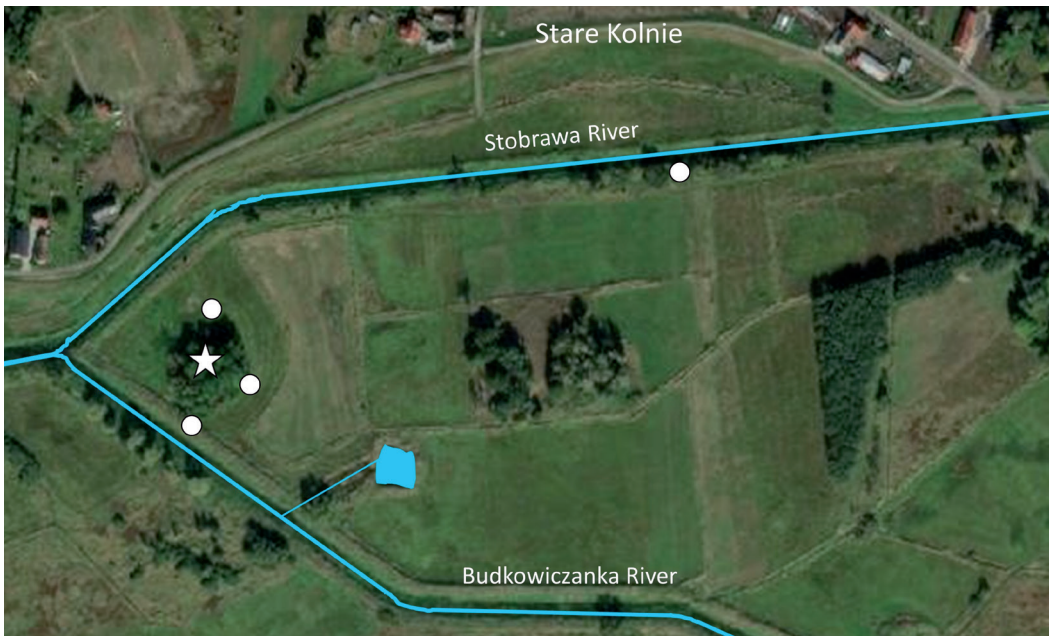

Fig. 1. A satellite map of the Castle Kolno area: the castle hill covered with trees (star) and blackthorn fruit collection points (white dots) at N and E edges of the hill and near Stobrawa and Budkowiczanka rivers (https://satelita.mapa.info.pl/#, modified)

found there. Most of the fossil stones were obtained from layers at the depth of 165-190 cm. Modern stone samples were collected in the vicinity of the castle ruins (Fig. 1).

Stones of *P. spinosa* were described based on four characters: stone length (L), stone width (W), height of ventral raphe (HVR), and W/L ratio. The characters were selected from a larger set of data reported by Staszak (2004) for blackthorn stones. Each stone was treated as an operational taxonomic unit (OTU) in numerical analysis and marked in diagrams and photos by a letter and a number. Fossil stones were marked as F1, F2, H1, H2, H3, H4, H5, H6, I1, K1, L1, L2, and M1, while modern ones as Ps1 to Ps20. Ps1 and Ps12 to Ps20 were collected near the Budkowiczanka River, and they are of putative hybrid origin. Specimens Ps19 and Ps20 were stones from unripe and dried fruits. The other Ps specimens were collected near the castle hill and near the Stobrawa River. The current analysis is complementary to a previous one (Kosina & Marek

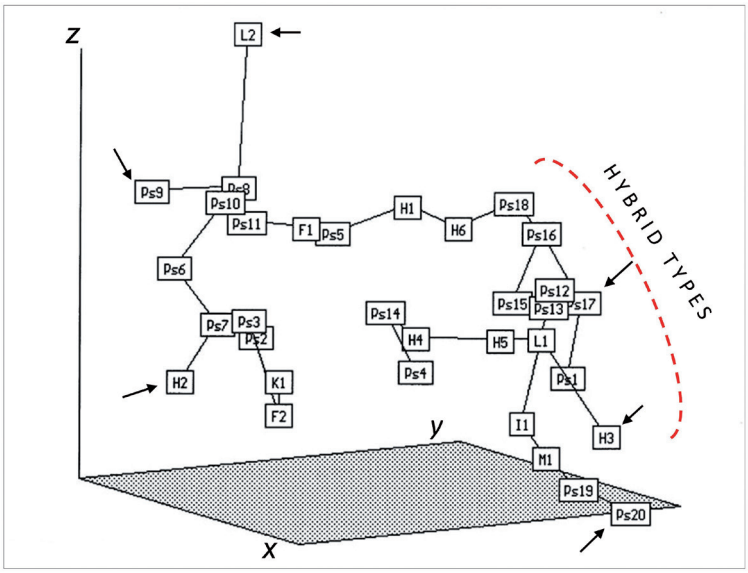

Fig. 2. Minimum spanning tree of the contemporary and fossil stones (OTUs) of *Prunus spinosa* in an ordination space (*x*, *y*, and *z* axes). OTUs were described by 4 traits of the stones. Extreme OTUs are marked by arrows. Putative hybrids between *P. spinosa* and domesticated plums are marked by a broken red line. Abbreviations are explained in Materials and methods

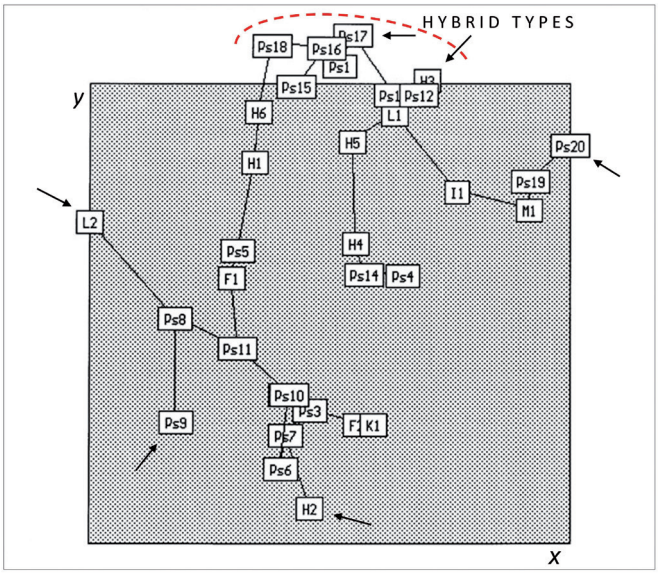

Fig. 3. A top view of the minimum spanning tree from Fig. 3. OTUs are viewed along ordination axes *x* and *y*

2021). The analysed collection of stones includes all fossil specimens and contemporary specimens collected in 2022 (Fig. 1). Compared to the previous analysis, the current one does not include specimens collected elsewhere in Poland. Thus, the current set represents one population with narrowed variability. In this population, more often vegetative reproduction (clones) than sexual reproduction was observed, with small production of fruits and seeds.

A matrix of average taxonomic distances between OTUs ($n = 33$) within a given set of OTUs was generated. This matrix was transformed into a configuration matrix by using the Kruskal method of nonmetric multidimensional scaling (nmMDS), and the configuration matrix was later applied to position the OTUs in a minimum spanning tree in a three-dimensional (x, y, z) coordination space (Figs. 2-3). Numerical analyses were performed using NTSYS software (Rohlf 1994).

3. Results and discussion

3.1. Correlation analysis

The analysed collection of 33 stones, derived from this locality, included both fossil and modern ones.

Table 1. Pearson's coefficients of correlation of stone characters of *Prunus spinosa* ($n = 33$)

Characters	L	W	HVR	W/L
L	1.00			
W	0.16	1.00		
HVR	0.14	0.33*	1.00	
W/L	-0.67***	0,62***	0.13	1.00

Explanations: *, *** – significance level at $\alpha = 0.05$, 0.001, respectively

Thus these stones are not from identical populations, although they may appear to be of the same origin. The previously analysed set (Kosina & Marek 2021) represented broader inter-stone variability, which may be characterised by several and more significant correlation coefficients. A significant correlation was then observed between the main stone dimensions: L and W. In the present study (Table 1), this coefficient is non-significant. The remaining significant coefficients clearly indicate the relationship between the development of the stone traits, but do not indicate inter-population genetic variability. Results of this analysis suggest that the medieval and present populations are morphologically similar.

3.2. Ordination analysis

The extreme OTUs in the minimum spanning tree (Figs. 2-3) were as follows: H2 (Fig. 4A) – small, round stone, shaped like cherry stones; H3 (Fig. 4B) – large stone, long with a sharp tip, fully developed, possible hybrid morphotype; L2 (Fig. 4C) – a stone with shallow reticulate sculpture, almost round, similar to the modern *Prunus domestica* subsp. *syriaca*; Ps9 (Fig. 4D) – a specimen from the castle hill, stone wider than high; Ps17 (Fig. 4E) – a specimen from a putative hybrid near the Budkowiczanka River, almost identical to the fossil specimen shown in Fig. 4B; Ps19 and Ps20 (Fig. 4F) – stones from fruits that died at an early stage of development due to drought: elongated, with a sharp tip, similar to H3 and Ps17.

The morphotype analysis of the leaves of short shoots (Fig. 5) showed that the putative hybrid had larger leaves, although it was morphologically similar to the morphotypes from the castle hill and from the site near the Stobrawa River. The leaves of this

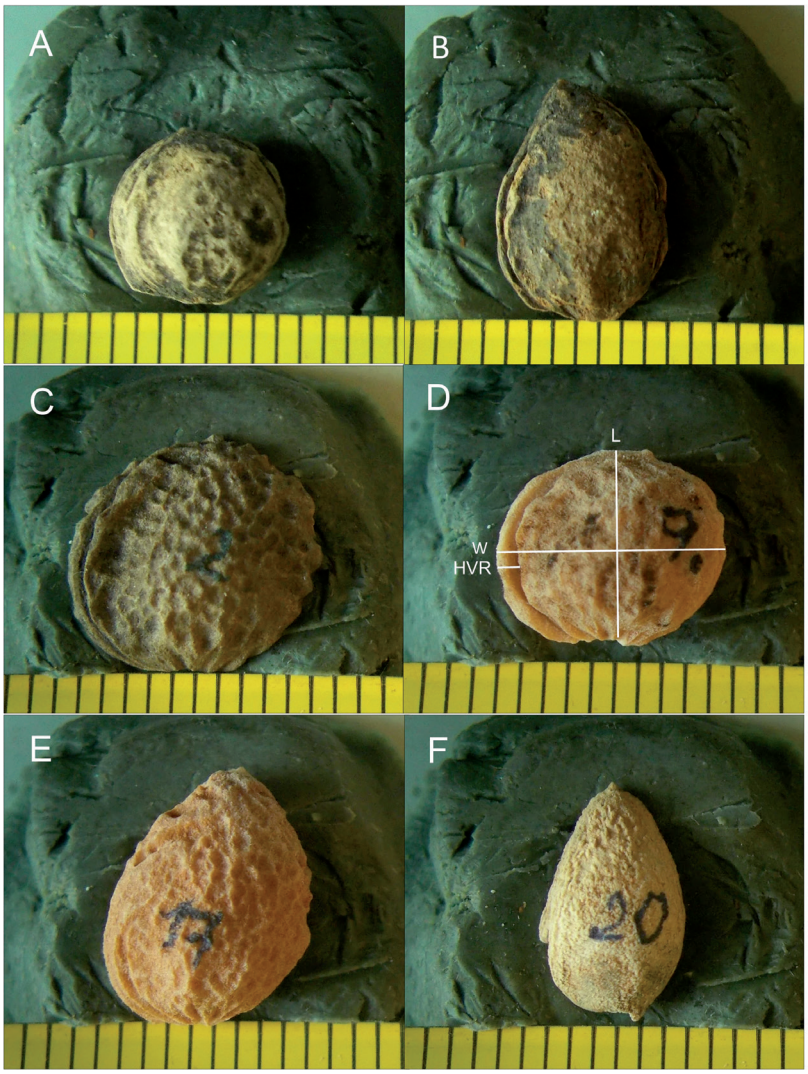

Fig. 4. Morphotypes of fossil and contemporary stones of *Prunus spinosa* occupying extreme positions in the minimum spanning tree (see Figs. 3 and 4)

Explanations: A – H2, B – H3, C – L2, D – Ps9, E – Ps17, F – Ps20. Measured traits of stone are marked in D. Scale in mm

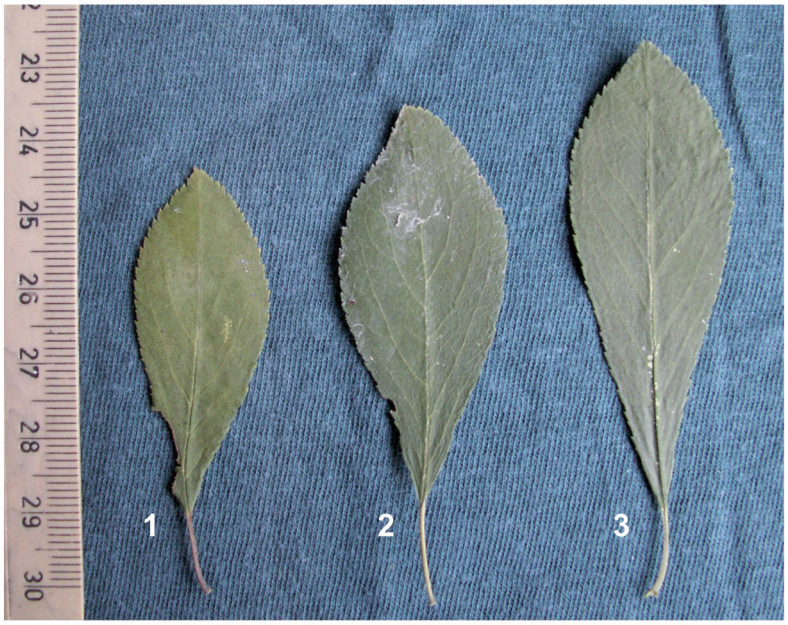

Fig. 5. Morphotypes of leaves of wild and hybrid forms of *Prunus spinosa* in the vicinity of Castle Kolno

Explanations: leaves collected, 1 – near the Stobrawa River; 2 – near the castle hill; 3 – a putative hybrid near the Budkowiczanka River. Scale in mm (cm)

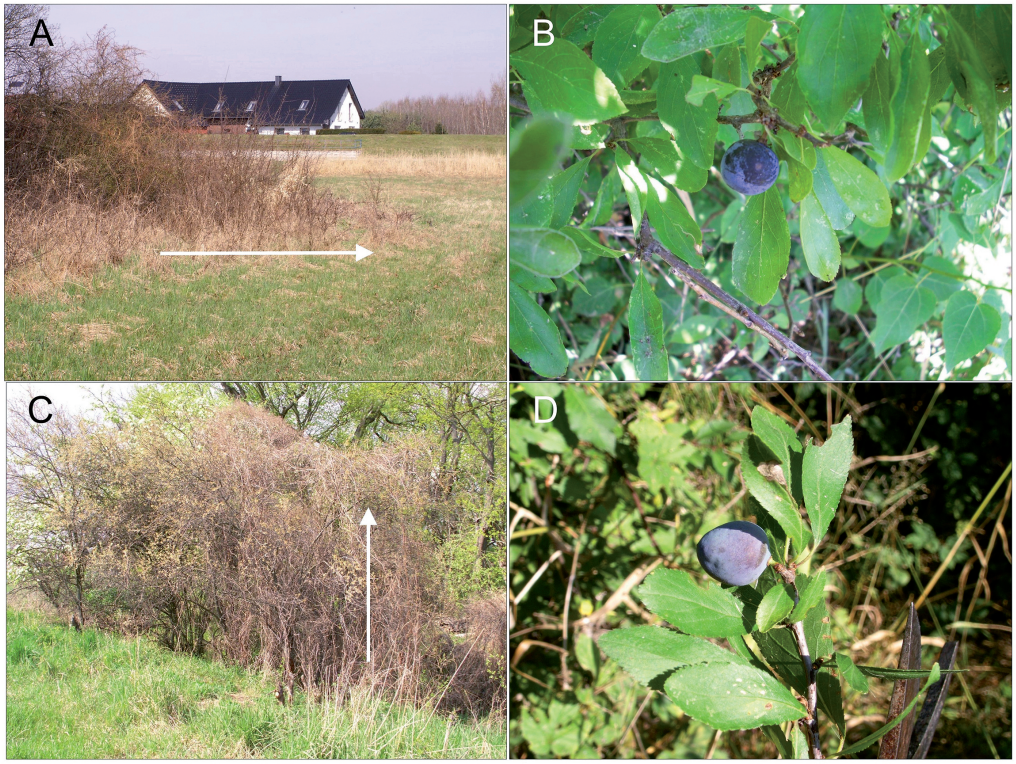

Fig. 6. Shrubs of *Prunus spinosa* in the vicinity of Castle Kolno

Explanations: A – on the N side of a hill with a clear clonal growth (arrow), B – a short shoot of a wild form near the Stobrawa River, C – a putative hybrid near the Budkowiczanka River, showing a clear vertical growth (arrow), D – a short shoot of the hybrid

possible hybrid type were not similar to the leaves of *P. domestica*. Moreover, the fruits of the hybrid type were larger and had longer pedicels (Fig. 6B, 6C) than those of the typical *P. spinosa*.

These findings indicate that in the vicinity of Castle Kolno, both in the Middle Ages (14th-15th centuries) and at present, the wild blackthorn bushes also include types with the characteristics of a hybrid between blackthorn and cultivated plums. The hybrid shrubs/trees are characterised by erect habit, weak clonal reproduction, and larger, elongated fruits. A specimen of the dried ripe fruit of the hybrid is approximately 13.5 mm long, with an approximately 3-mm-long pedicel. These dimensions are below those reported for *Prunus ×fruticans* by Kühn (Kühn 1988). Hanelt (1997) indicated difficulties in differentiating hybrids of *P. spinosa* because of the high variability within the species. Fossil types reported by van Zeist *et al.* (1994) and described as *P. spinosa* var. *macrocarpa* vary in size and shape: they are round or ovoid, but without the conical, sharp tips similar to those of *P. domestica*. A slightly greater variability of fossil blackthorn stones from a site near Heidelberg from the 13th century was described by Körber-Grohne (1979). For large medieval samples of *P. spinosa* from Lübeck and modern var. *macrocarpa* from Schleswig-Holstein, the variability of the stone features of the modern form showed a broad distribution, while that of the fossil form showed a narrow one (Kroll 1980). This finding may suggest recombinational variation and hybrid origin of the modern var. *macrocarpa*.

It should be emphasised that distinct hybrid types in the area around Wrocław (SW Poland) were found near former settlements or existing plum plantations (Staszak 2004). Since the spectrum of subspecies and cultivars of *P. domestica* is wide and cross-breeding with *P. spinosa* is a sporadic but constant process over time, the hybrids have various morphotypes. A fossil type classified by Pollmann *et al.* (2005) as *P. insititia*/*spinosa* from the 3th century A.D., from a Roman settlement in Switzerland, was almost identical to the modern type shown in Fig. 4D, which is similar to the fossil '*syriaca*' specimen shown in Fig. 4C.

The modern populations of *P. spinosa* show a large variability of traits but the traits of stones were considered as stable and valuable for taxonomic analysis (Hübner & Wissemann 2004; Staszak 2004). Covariance analysis of the characteristics of stones and leaves of this species confirmed that the characteristics of both organs can be used independently in such analyses (Kosina 2005).

A detailed morphological and phenological analysis (Vander Mijnsbrugge *et al.* 2016) and breeding tests (Woldring 1997/1998) of the *P. spinosa* – *P. ×fruticans* complex showed that the latter taxon is certainly a hybrid

of blackthorn and cultivated plums as a result of various crossings. The clear uniparental dominance of traits in the principal components analysis (PCA) diagram for Danish populations of *P. spinosa* and *P. domestica* subsp. *insititia* indicated that backcrosses of putative hybrids occurred more frequently with *P. domestica* (Nielsen & Olrik 2001). According to Grant (1981), these data reinforce the view that the wild-cultivated plums complex is a dynamic syngameon in the speciation process (Kosina & Marek 2021). The stigma-pollen responses between *P. spinosa* and *P. cerasifera* (Staszak 2004) justify the inclusion in the syngameon of plums also the latter species, present in Europe since the early Middle Ages.

4. Concluding remarks

Fossil forms from the Middle Ages and contemporary *P. spinosa* stones collected near the medieval Castle Kolno indicate the presence of hybrid types originating from spontaneous crossings with domestic plums in this area. These stones can be related to the taxonomically recognised type *P.* ×*fruticans*. Correlation analysis of stone features suggest that these dependencies are developmental and are already manifested in immature forms. The pattern of OTU variation in the ordination space for a narrow local population corresponds well to that of the syngameon component. Plum orchards probably existed in the nearby settlement of Stare Kolnie in the Middle Ages, which allowed crossings between the cultivated plums and blackthorn. The determination of whether the recognised putative hybrid near the ruins of the castle is identical to fossil stones requires molecular DNA analysis, and the effectiveness of such analyses depends on the state of preservation of DNA in fossil remains.

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