Babeș -Bolyai University
Faculty of Biology and Geology
Integrative Biology doctoral school

DOCTORAL THESIS

Comparing ecology and phenotypic plasticity of Romanian hawthorn (*Crataegus* L.) species and their hybrids

-SUMMARY-

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Keywords

Crataegus L., hybridization, introgression, morphometry, determination keys, phenotypic plasticity, shade tolerance, performance, allocation, phenology, niche breadth, niche overlap, habitat fragmentation, species distribution models,

Summary of the thesis

Chapter I. – General introduction into hawthorn taxonomy, biology and ecology

Parts of Chapter I were published in: **Kuhn T.**, Jancsó B. and Ruprecht E. (2020). Hawthorn (*Crataegus* L.) taxa and their hybrids in North-Western Romania: a recommendation for national identification keys based on morphometric analysis. *Contribuții Botanice* 55, 7–26.

Hawthorns (*Crataegus* L.) are a diverse genus of shrubs and small trees, widespread and common throughout the Northern hemisphere (Christensen, 1992). Hybridization between mostly diploid species is common within the genus, and hybrids are often polyploid (Talent and Dickinson, 2005). Furthermore, while species are obligate outcrossers and self-incompatible, hybrids are pseudogamous apomicts, and reproduce asexually (Vašková and Kolarčik, 2019). Introgression towards one of the progenitor species further blurs species boundaries, making identification difficult (Christensen, 1992). Hawthorns have remarkable ecological and economical significance. They represent important food sources and shelter for various animals and play a crucial role in successional processes. Furthermore, hawthorns are important components of hedges and hedge-banks, and are commercially exploited for their medicinal, nutritional, and decorative properties (Fichtner and Wissemann, 2021, Thomas et al., 2021). Thus, numerous scientific studies explore their biology, genetics, biochemistry, and ecology. However, while these aspects are well studied in North-America, where hawthorn diversity is the highest, numerous aspects of the ecology and biology of European hawthorn species remain less explored (Talent and Dickinson, 2005). Nonetheless, hybrids are well documented to be common, and regionally even more abundant relative to their progenitors, occurring outside the hybrid zone (Christensen, 1992). Various hypothesis have been suggested to explain the high abundance of hybrids in natural habitats, with the dominant one being, that land use intensification and habitat fragmentation increases hybridization odds between sympatric species (Christensen, 1992, Oklejewicz et al., 2013). Considering this, in the present thesis we focus on exploring the potential ecological and biological driving factors behind hybrid success, in the case of hybridizing temperate-European hawthorn species.

- Our study had the following objectives:

(**A**). In our “pilot” study, we investigated the morphology of *Crataegus* species and their hybrids from North-western Romania, and compiled national identification keys for the genus (**Chapter II** of the thesis).

(**B**). Comparing the performance, allocation patterns and phenotypic plasticity of the naturally occurring and widespread hybrid *Crataegus* × *subsphaerica* (*C. monogyna* × *C. rhipidophylla*)’s seedlings to that of its progenitors in a controlled common garden experiment (**Chapter III** of the thesis).

(**C**). Exploring and comparing the flowering phenology of three sympatric *Crataegus* species (*Crataegus laevigata, C. monogyna, C. rhipidophylla*) and their natural hybrids (*C.* × *subsphaerica, C.* × *media, C.* × *macrocarpa*), in order to get an insight into the formation and survival of *Crataegus* hybrids (**Chapter IV** of the thesis).

(**D**). Comparing the niche breadth and niche overlap between hybrids and their progenitor taxa by multiple niche parameter groups, defined based on macro- and microenvironmental variables (**Chapter V** of the thesis).

(**E**). Comparing the environmental requirements and distribution patterns of hawthorns and their hybrids in North-western Romania, based on field survey data, and using random forest models and species distribution models (**Chapter VI** of the thesis).

CHAPTER II. - Hawthorn (*Crataegus* L.) taxa and their hybrids in north-western Romania: a recommendation for national identification keys based on morphometric analyses

Chapter II was published in: **Kuhn T.**, Jancsó B. and Ruprecht E. (2020). Hawthorn (*Crataegus* L.) taxa and their hybrids in North-Western Romania: a recommendation for national identification keys based on morphometric analysis. *Contribuții Botanice* 55, 7–26.

Crataegus species are common and widespread shrubs or small trees across Europe, and are of considerable ecological and agricultural importance. However, controversy still surrounds the taxonomy and ecology of this genus, mainly due to frequent hybridization between sympatric species and introgression of genes (Christensen, 1992). Considering the poor representation of *Crataegus*-related research in the Romanian botanical literature, we investigated the occurrence and morphology of native *Crataegus* species and their putative hybrids in north-western Romania.

We collected herbarium specimens from three regions (Zărandului Mountains, Transylvanian Plain, Călata region), from a variety of habitats, including forests, grasslands and forest-grassland ecotones. Morphometric measurements were taken on a total of 34 fruit-, leaf- and stipule characters on generative shoots. We used PCA and Random Forest analysis to select for the 'best' characters differentiating between species and hybrids.

We identified ten different *Crataegus* taxa, including species, subspecies, varieties, and their hybrid nothotaxa (Appendix I.): *Crataegus monogyna* (mainly in grasslands and other open habitats), *C. rhipidophylla* var. *rhipidophylla, C. rhipidophylla* var. *lindmannii* and *C. laevigata* subsp. *laevigata, C. laevigata* subsp. *palmstruchii* (mainly in forests), *C.* × *kyrtostyla* nothovar. *domicensis*, *C.* × *media*, *C.* × *macrocarpa* nothovar. *macrocarpa* and *C.* × *macrocarpa* nothovar. *hadensis* (mainly in forest openings and near forest edges). The most common taxa identified was *C.* × *kyrtostyla* nothovar. *kyrtostyla* (common in grasslands, rarer in forests).

The best differentiating characters between species and hybrids were (1) number of styles, (2) fruit length, (3) number of teeth on stipules, (4) position of sepals on mature fruits, (5) number of serrations on basal leaf lobe, (6) ratio between serrate part of lower leaf lobe and the entire part, (7) depth of basal sinus, (8) and ratio between sepal length and width. Based on the results of the morphometric analysis and literature review, we constructed national identification keys of all *Crataegus* taxa identified in this study and other confirmed taxa present in Romania. Additionally, we have illustrated the most common six identified taxa (including hybrids).

CHAPTER III. - Seedling performance, allocation patterns and phenotypic plasticity of two sympatric hawthorn species and their natural hybrid

Chapter III was published in **Kuhn, T.**, Györfi, O., Ruprecht, E. (2022). Seedling performance, allocation patterns and phenotypic plasticity of two sympatric hawthorn species and their natural hybrid. *Flora* 287, 151994

Hybridization followed by introgression is common between European *Crataegus* species, while hybrids of numerous sympatric taxa are abundant and widespread in several natural and seminatural environments. However, no study to the present day has compared the development of *Crataegus* species and their hybrids in their seedling stage. Our aim was to compare seedling performance, biomass allocation and phenotypic plasticity of two sympatric *Crataegus* species (*C. monogya* and *C. rhipidophylla*) with contrasting shade tolerance and their natural hybrid along light and water availability gradients in a controlled pot experiment. By this means we tested which of the existing hybrid zone models (Arnold, 1997) can successfully explain the field distribution and habitat preference of the hybrid.

We hypothesized that under lower levels of light, shade tolerant *C. rhipidophylla* will have a greater biomass production, greater leaf mass fraction, denser stems and lower root mass fraction relative to the shade intolerant *C. monogyna*, which in turn will have a higher biomass production under ambient light conditions. The hybrid is expected to be intermediate in trait responses relative to its progenitors under all light availability levels. Furthermore, under low levels of water availability, *C. monogyna*, as a grassland specialist, is expected to have a greater biomass production, due to greater root mass fraction relative to *C. rhipidophylla*, while the hybrid is expected to be intermediate in these. In addition, *C. rhipidophylla* is expected to be more conservative in trait responses to the levels of water availability but more plastic in response to light availability relative to *C. monogyna*, while we expect the hybrid to be intermediate in phenotypic plasticity between its progenitors (Valladares and Niinemets, 2008).

The performance of both species and their hybrid decreased in deep shade as well as under high water availability. Variation in light availability had a more pronounced effect on seedling development than that in water availability. These results evidenced, that habitat differentiation between the two species under natural conditions is probably mainly driven by competitive interactions. The shade tolerant forest species *C. rhipidophylla* had shorter, more dense stems compared to the shade intolerant *C. monogyna*, with the hybrid being intermediate in these characters. This suggests a steadier growth and higher resource allocation into stems in the case of the shade tolerant species, which is in accordance with the shade-tolerance syndrome that states, that shade tolerant species are less competitive under ambient light conditions relative to shade intolerant species (Valladares and Niinemets, 2008).

Furthermore, the hybrid was phenotypically more plastic in biomass allocation relative to at least one of its progenitor species under various water availabilities, suggesting an advantage on behalf of the hybrid in establishment under a wider range of environmental conditions. Our results support the “mosaic-model” that describes most accurately the hybrid zone.

CHAPTER IV. - Flowering phenology may shape hybridization patterns of hawthorn (*Crataegus* L.) species

Chapter IV was published in: **Kuhn, T.** and Ruprecht, E. (2022). Flowering phenology may shape hybridization patterns of hawthorn (*Crataegus* L.) species. *Contribuții Botanice* 57, 95-107.

Asynchronous flowering phenology is an important prezygotic barrier to hybridization, especially in the case of sympatric species (Rieseberg, 2007), while the degree of overlap in flowering can influence hybridization odds and shift introgression. *Crataegus* species have protogynous flowers, with female sexual organs reaching maturity earlier than male sexual organs, thus, the likelihood of interspecific pollen transfer from earlier flowering (being the pollen donor) towards later flowering species (being the mother plant) may shift introgression, favouring later flowering species as the maternal progenitor.

In our study, we investigated the flowering phenology of three sympatric *Crataegus*-species and their hybrids in Transylvania, Romania. The methodology was of phenological scoring and statistical analysis was largely based on Mijnsbrugge et al. (2015). He hypothesized, that progenitor species of more commonly occurring natural hybrids will overlap more in their flowering phenology, while hybrids will be intermediate relative to their progenitors in their phenology.

Our results revealed, that *C. laevigata* flowered first at the end of April, followed by *C. rhipidophylla* and the hybrid taxa *C.* × *subsphaerica* and *C.*× *media*, *C.* × *macrocarpa* 8 days later, while *C. monogyna* flowers last, 3 days later. The progenitor species of the most frequent hybrid taxa *C.* × *subsphaerica* have been found to have the greatest overlap in their flowering, while hybrids of *C. laevigata*, which overlaps narrowly in its flowering with the other two species, are rare. Interestingly, all three hybrid taxa overlapped almost perfectly in their flowering phenology with that of *C. rhipidophylla*, and except for *C.*× *media*, hybrid taxa are not intermediate in the timing of their flowering relative to their progenitors.

Our results provide evidence that distribution patterns and frequency of *Crataegus*-hybrids in the landscape are at least partially shaped by the progenitor species' overlap in flowering phenology, which influences hybridization odds and may shift introgression towards one of the progenitor species.

CHAPTER V. - Niche breadth and overlap of pseudogamous apomictic hawthorn (*Crataegus* L.) hybrids and their progenitors

Manuscript under review at *Preslia*: **Kuhn, T.** and Ruprecht, E. (2023). Niche breadth and overlap of pseudogamous apomictic hawthorn (*Crataegus* L.) hybrids and their progenitors

Hybridization followed by introgression is well documented within the *Crataegus* genus, and while many hybrids are known to be frequent and widespread, factors influencing gene flow are still poorly understood. In this study, we compare niche breadth and overlap between hybrids and their progenitors and investigate ecological factors potentially influencing hybrid and progenitor distribution and frequency.

We hypothesized, that hybrids will have relatively wider niches to progenitors, and hybrids will overlap more with their progenitors in their niche, that the progenitors with each other. Furthermore, we assumed that niche breadth of the hybrid taxa and progenitor species will also correlate with their range size and abundance (Blaine Marchant et al., 2016). In the case of ecological factors, we assumed hybrid intermediacy relative to progenitors.

Based on our field investigation, most *Crataegus* hybrids were relatively rare compared to their progenitors, except *Crataegus × subsphaerica* (*C. monogyna × C. rhipidophylla*), which was the most common taxa in the study area, being present even outside of the hybrid-zone, suggesting range expansion. Furthermore, it seems that the distribution of the studied *Crataegus* taxa is determined primally by climatic factors and light availability, and they appear to be habitat generalists across different broad-leaved forest or grassland plant associations. Our results also revealed that most hybrids had progenitor-like niche breadths, and rare hybrids had surprisingly wide niche estimates, suggesting that hybrid frequency is influenced more by phenological overlap between progenitors and effective long-distance dispersal of apomictic seeds, rather than environmental selection against hybrids. In addition, the presumed intermediacy in ecological preferences of the studied *Crataegus* hybrids relative to progenitors was not as evident as we expected, probably due to introgression towards one of the progenitors.

Considering the frequent occurrence of hybrids outside the hybrid zone, and the mosaic distribution pattern of the habitat of sympatric species, a combination of the mosaic and evolutionary novelty model describes best the hybrid zone of the studied species (Arnold, 1997).

CHAPTER VI. - Comparing the ecology and distribution of hawthorn species and their hybrids through species distribution modelling

Manuscript in preparation: Kuhn, T. and Ruprecht, E. Comparing the ecology and distribution of hawthorn species and their hybrids through species distribution modelling.

Species distribution models are a widely used statistical tool by ecologists to predict and explore geographical range and abundance patterns of plants and animals, based on environmental characteristics and occurrence observations. In this study, we explored and compared through this method the distribution, abundance, and environmental requirements of hawthorn hybrids and their parental progenitors based on a field investigation in the North-western part of Romania. We hypothesized, that hybrids will be more common, where the distribution and environmental requirements of progenitor species overlap. Furthermore, we assumed that hybrids between forest and grassland species will be more frequent in mosaic landscapes, while the distribution of hybrids between forest species will follow landscapes dominated by forests. In addition, we expected hybrid morphotypes with intermediate characters between their progenitors will appear in the contact zone (hybrid-zone) of progenitor species and manifest also intermediate ecological behaviour, while parent-like morphotypes will resemble more to their progenitor species in their distribution and ecological requirements.

Our results revealed that climatic factors were the most important, while forest extent the second most important predictors for the distribution and abundance of hawthorn taxa. Hawthorns were most common at mid elevations in the foothills, and were sporadic or absent from the mountainous belt upwards. They also clustered mostly in the proximity of forest ecosystems. In most of the cases, hybrids resembled to one of their progenitors in their environmental requirements and distributional range, presumably due to introgression from hybrids genotypes towards progenitors. However, morphologically intermediate *Crataegus* × *subsphaerica* hybrids (*C. monogyna* × *C. rhipidophylla*) occupied also environmentally intermediate niches relative to their progenitors, and were most common in highly fragmented landscapes consisting of a mosaic of the two progenitor species' habitats (i.e. grasslands and forests). These results confirm the importance of anthropogenic landscape-fragmentation as a driver of interspecific hybridization between hawthorns, which was confirmed partially by other studies (Oklejewicz et al., 2013). Furthermore, the presence or even high abundance of some hybrid taxa (*Crataegus* × *subsphaerica, C.* × *media, C.* × *kyrtostyla*) outside the distributional range of one or both progenitor species confirms the spread of mostly introgressed hybrid genotypes outside of the hybrid zone.

The observed distribution patterns for hybrids can be explained by (1) the divergence of flowering phenology between species, where the most common hybrids have progenitors with less diverging phenologies; (2) the abundance and intermixing likelihood of progenitor populations or specimens; (3) the high fitness, high phenotypic plasticity and effective dispersal ability of mostly polyploid hybrid genotypes due to their pseudogamous apomictic breeding system relative to their mostly diploid, obligate out-crosser progenitor species.

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Appendix I: Photos of hawthorn taxa occuring or potentially occuring in Romania

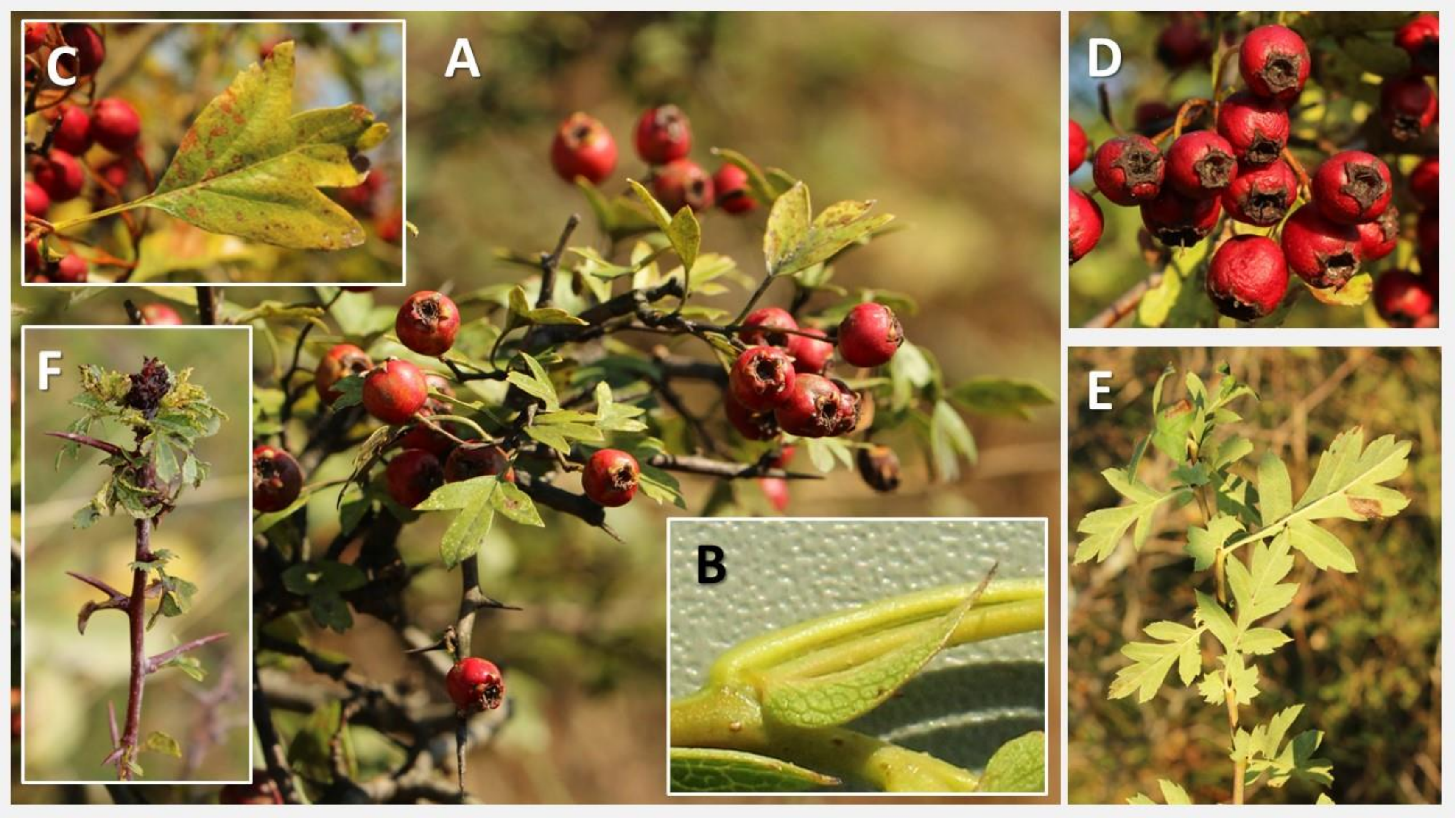


Figure 1 (A-E): *Crataegus monogyna* Jacq.; **A** – fruiting branch; **B** – stipules of generative shoot; **C** – subterminal leaf of generative shoot; **D** – pomes with sepals; **E** – sterile long shoot; **F** – *Crataegus monogyna* subsp. *brevispina* (Kunze) Franco, sterile elongate shoot; (photos: Kuhn Thomas, near Orșova (A), Cluj-Napoca (B - E) and Simeria (F), Romania)

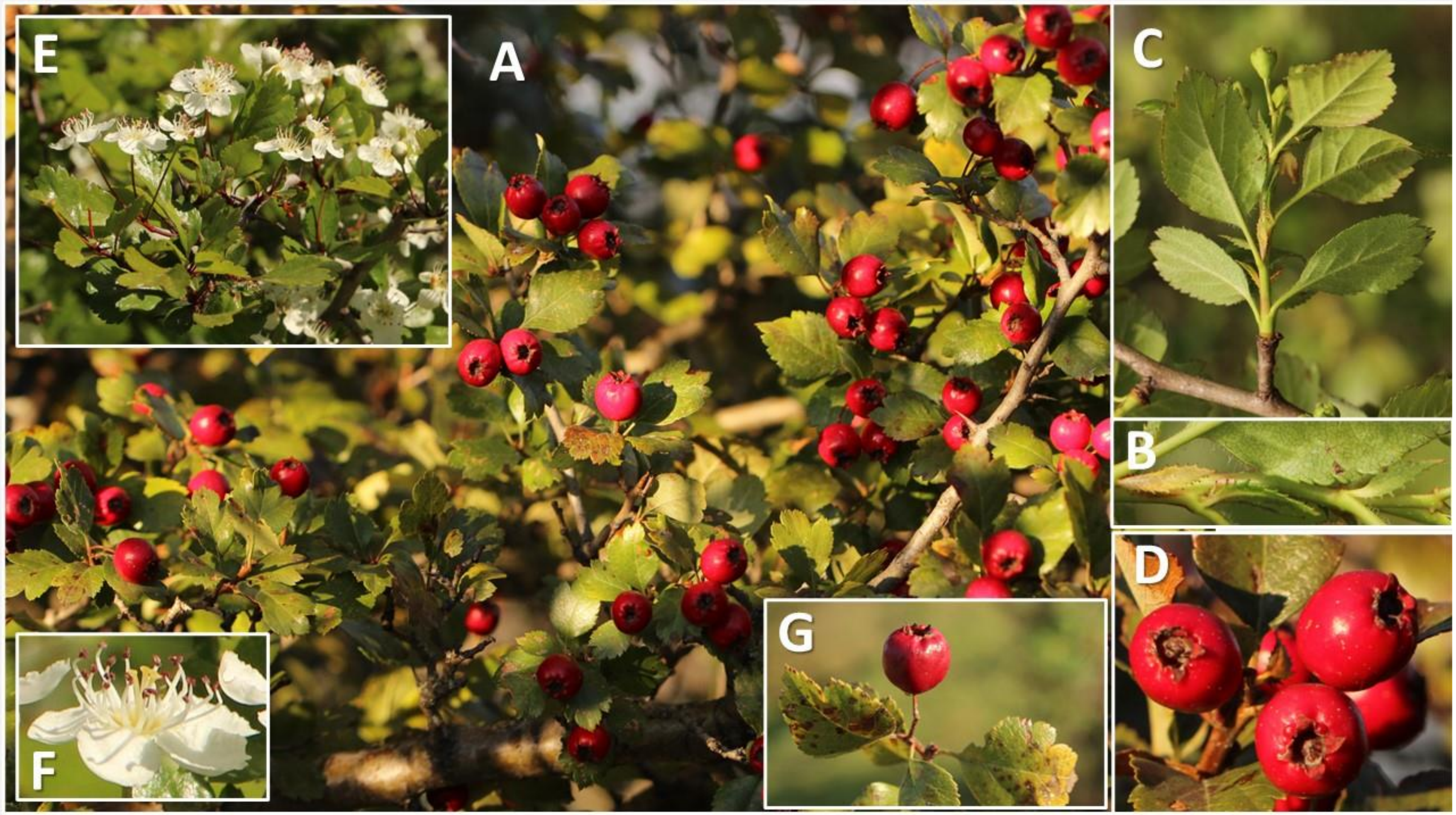


Figure 2 (A-F): *Crataegus laevigata* subsp. *laevigata* (Poir.) DC.; **A** – fruiting branches; **B** – stipules of the leaves of flowering shoots; **C** – abaxial surface of generative shoot with leaves, their stipules, and flowers before anthesis; **D** – pomes with sepals and styles; **E** – flowering branch; **F** – flower; **G -** *Crataegus laevigata* subsp. *palmstruchii* (Lind.) Franco, generative shoot; (photos: Kuhn Thomas, near Huedin (A, B, C, D), Beiuș (E, F) and Cluj-Napoca (G), Romania)

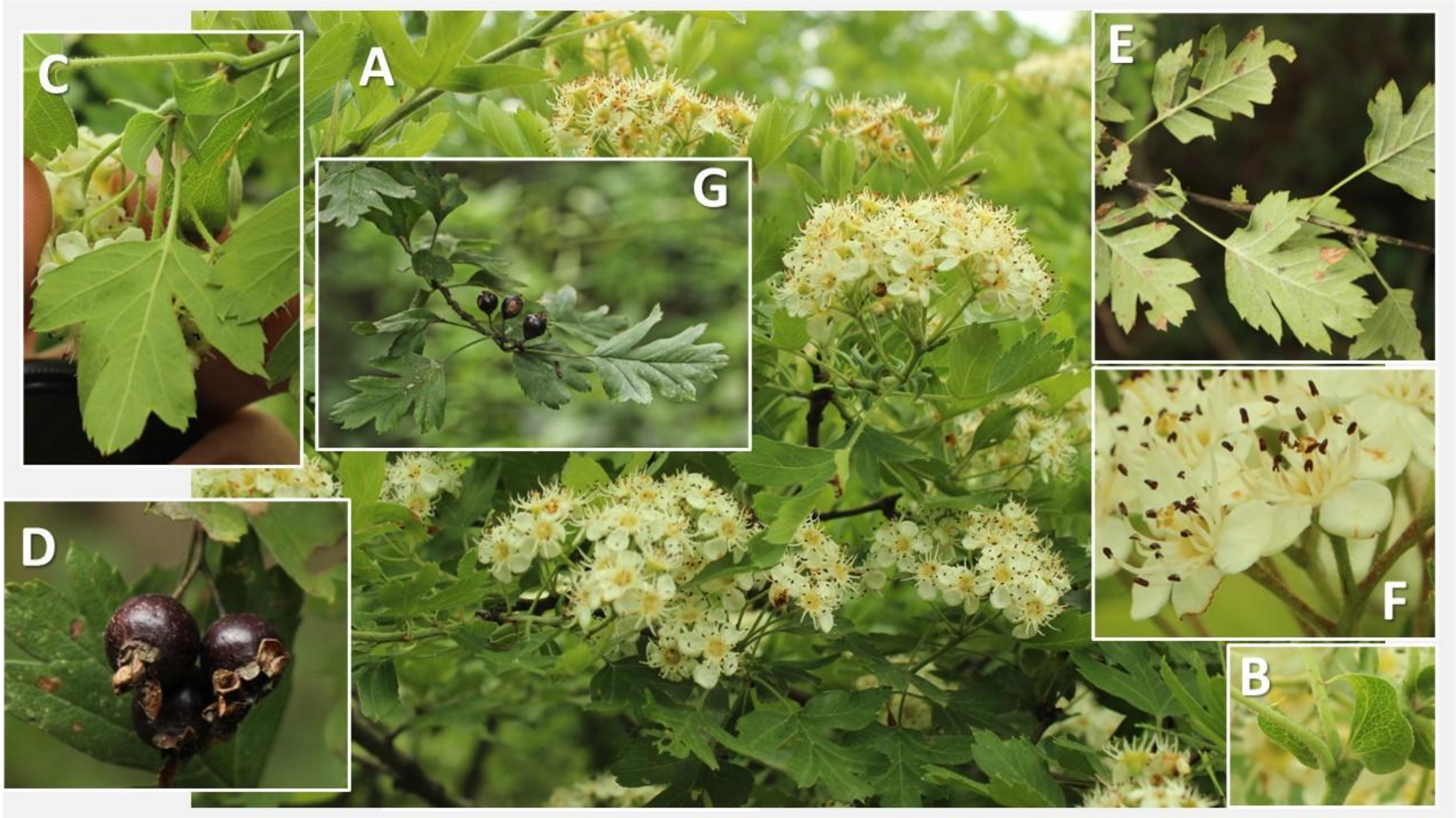


Figure 3 (A-F): *Crataegus pentagyna* subsp. *pentagyna* Wild.; **A** – flowering branches; **B** – stipule of leaf of generative shoot; **C** – abaxial sufrace of leaf of flowering shoot; **D** – pomes; **E** – sterile long shoot with leaves and their stipules; **F** – flowers; **G** – *Crataegus* × *rubrinervis* Lange, fruiting shoot; (photos: Kuhn Thomas, near Măcin (A, B, C, F) and Orșova (D, E, G), Romania)

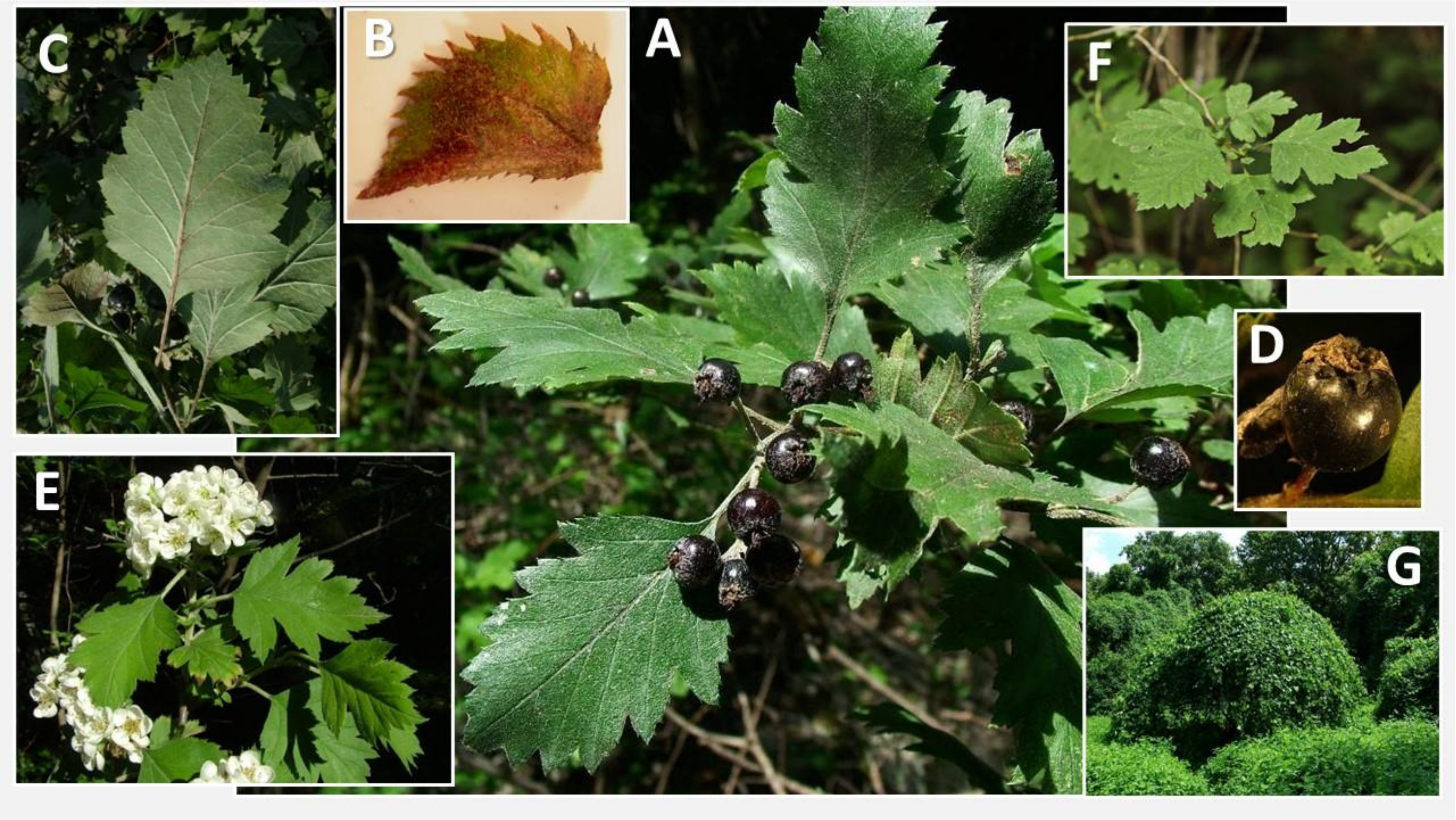


Figure 4 (A-G): *Crataegus nigra* Waldst. et. Kit.; **A** – fruiting branch; **B** – stipule of leaf of generative shoot; **C** – abaxial surface of leaf of generative shoot; **D** – pome; **E** – generative shoot with flowers; **F** – sterile long shoot with leaves and their stipules; **G** – habitus of specimen in its natural habitat (photos: Kerényi-Nagy Viktor, A, B, D, E, G – Szigetújfalu, C – Sükösd, Hungary; Kuhn Thomas, F – Insula Calinovăț – Porțile de Fier, Romania)

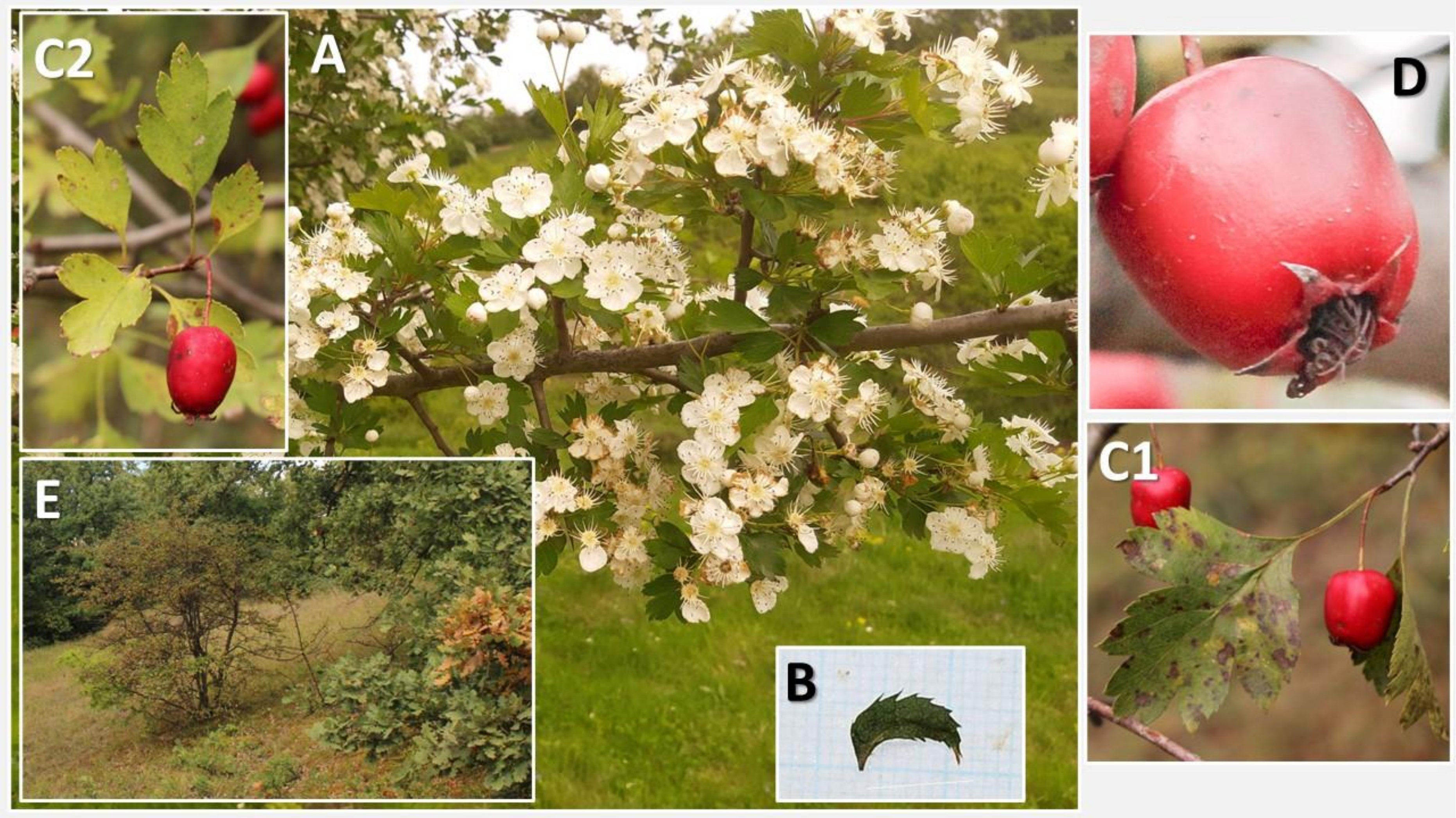


Figure 5 (A-E): *Crataegus rhipidophylla* var. *rhipidophylla* Gand. (syn.: *C. rosaeformis* Janka); **A** – flowering branch; **B** – stipule of leaf of generative shoot; **C1, C2** – generative shoots with pomes and leaves; **D** – pome with sepals; **E** – habitus of a specimen in its natural environment; (photos: Kuhn Thomas, near Cluj-Napoca, Romania)

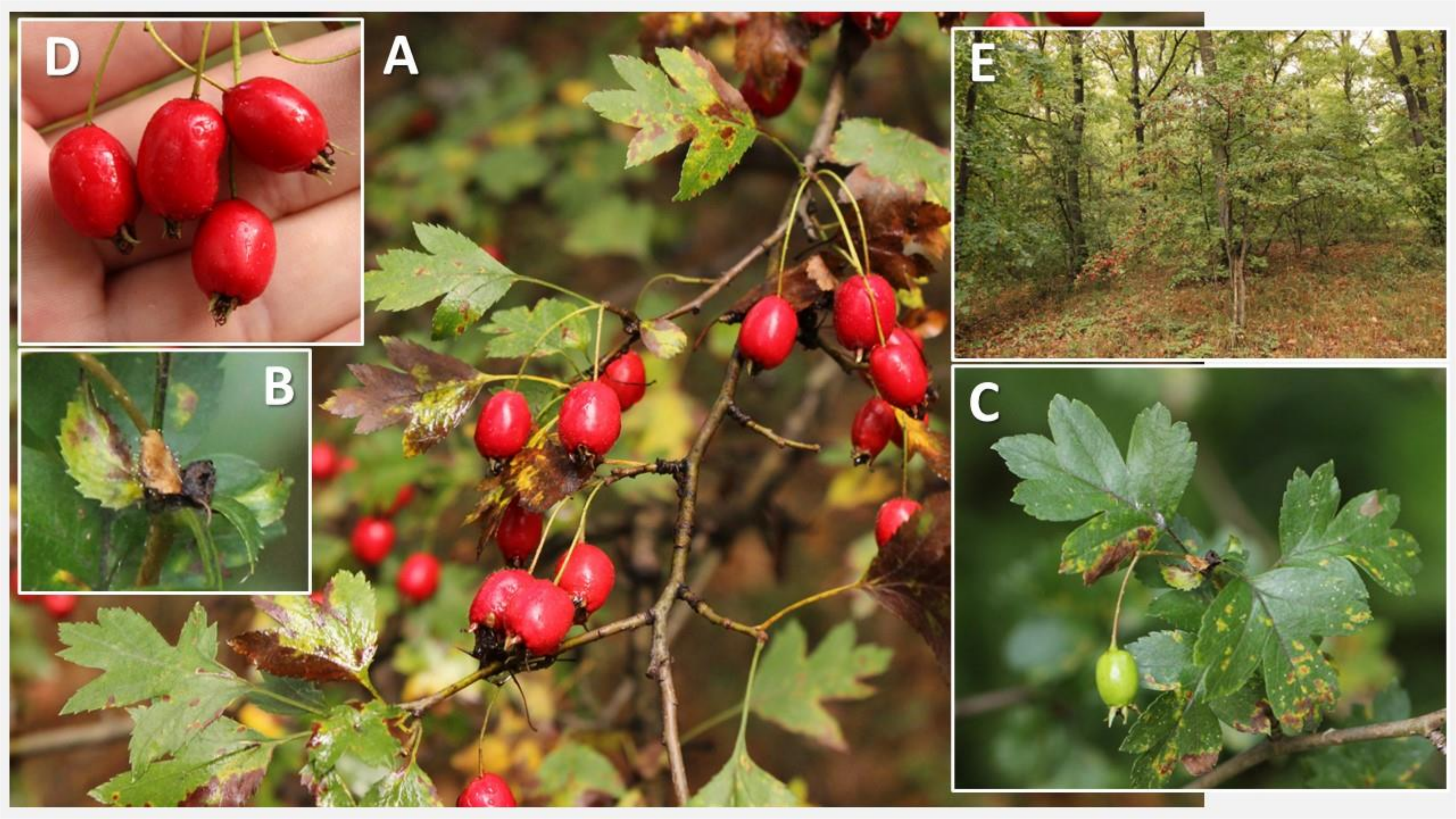


Figure 6 (A-E): *Crataegus rhipidophylla* var. *lindmanii* (Hrabětová) K.I. Christensen (syn.: *C. lindmanii* Hrab. – Uhr.); **A** – fruiting branches; **B** – stipules of the leaves of generative shoot; **C** – generative shoot with pome, leaves and their stipules; **D** – pomes and their sepals; **E** – habitus of a specimen in its natural environment; (photos: Kuhn Thomas, near Cluj-Napoca, Romania)

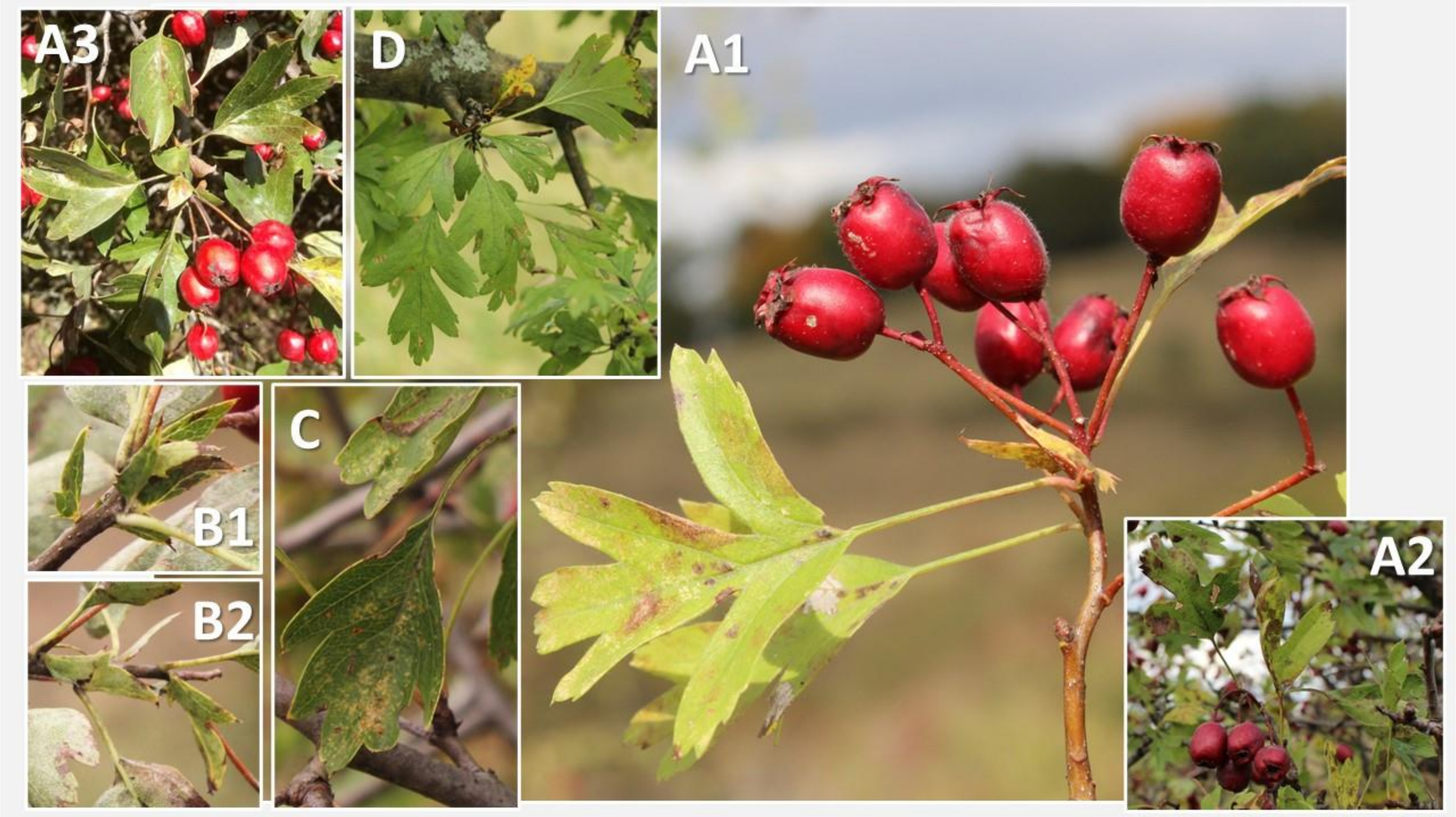


Figure 7 (A-D): *Crataegus* × *subsphaerica* Gand. (syn.: *C.* × *kyrtostyla* nothovar. *kyrtostyla* Fingerh.; *C. monogyna* × *C. rhipidophylla*); **A1, A2, A3** – generative shoot with pomes, leaves and their stipules; **B1, B2** – stipules of leaves of generative shoots; **C** – subterminal leaf of generative shoot; **D** – sterile short shoot; (photos: Kuhn Thomas, near Cluj-Napoca, Romania)

Figure 8 (A-C): *Crataegus* × *kyrtostyla* Fingerh. (syn.: *C.* × *kyrtostyla* nothovar. *domicensis* (Hrabětová-Uhrova) K.I. Christensen; *C. monogyna* × *C. lindmanii*); **A** – fruiting branches; **B** – stipules of generative shoot; **C1, C2, C3** – generative shoots with pomes, leaves and their stipules; (photos: Kuhn Thomas, near Gherla (A, B) and Cluj-Napoca (C1, C2, C3), Romania)

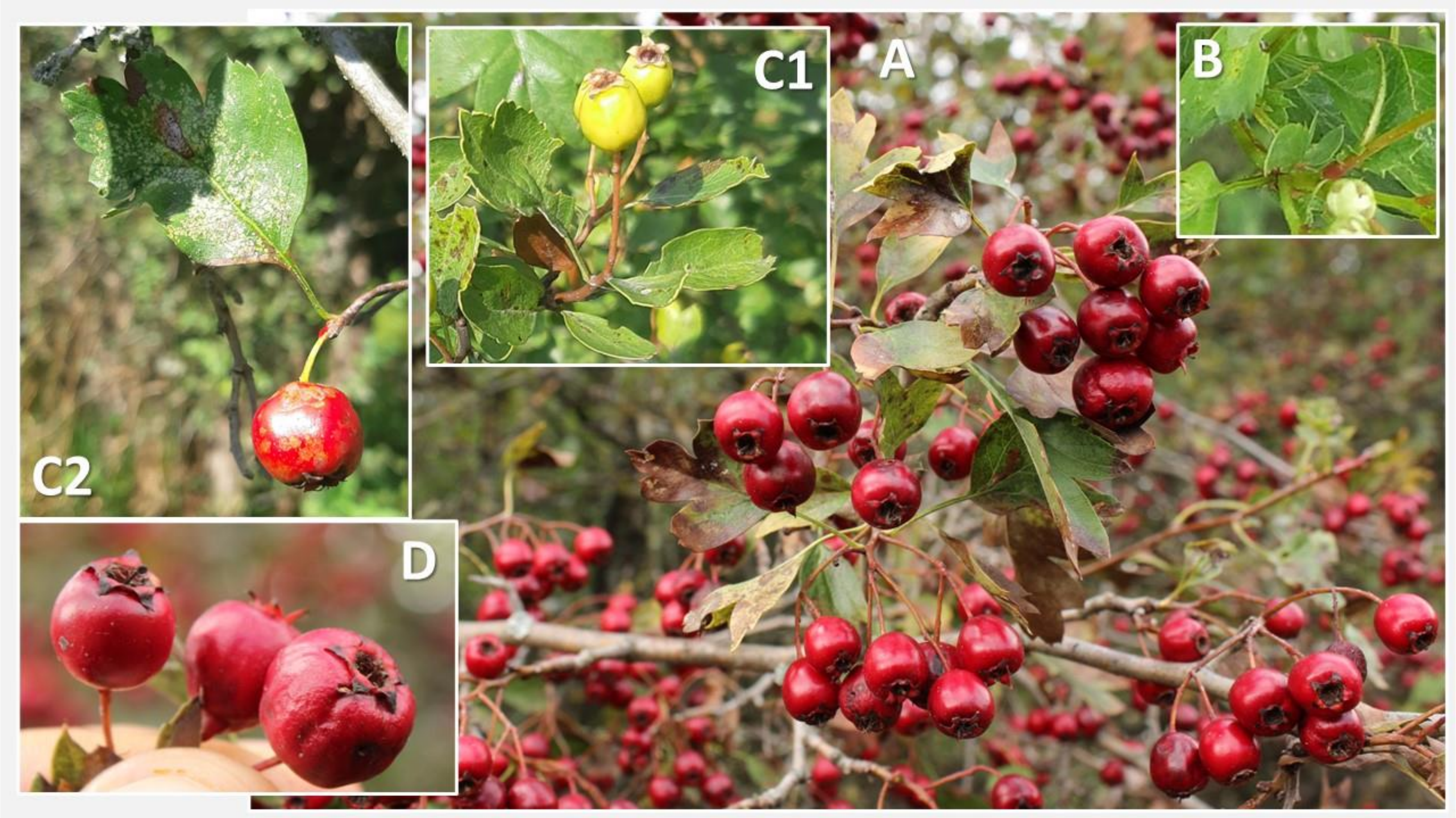


Figure 9 (A-D): *Crataegus* × *media* Bechst. (*C. monogyna* × *C. laevigata*); **A** – fruiting branch; **B** – stipules of leaves of a generative shoot; **C1, C2** – generative shoot with pomes and leaves; **D** – pomes with sepals and styles; (photos: Kuhn Thomas, near Cluj-Napoca, Romania)

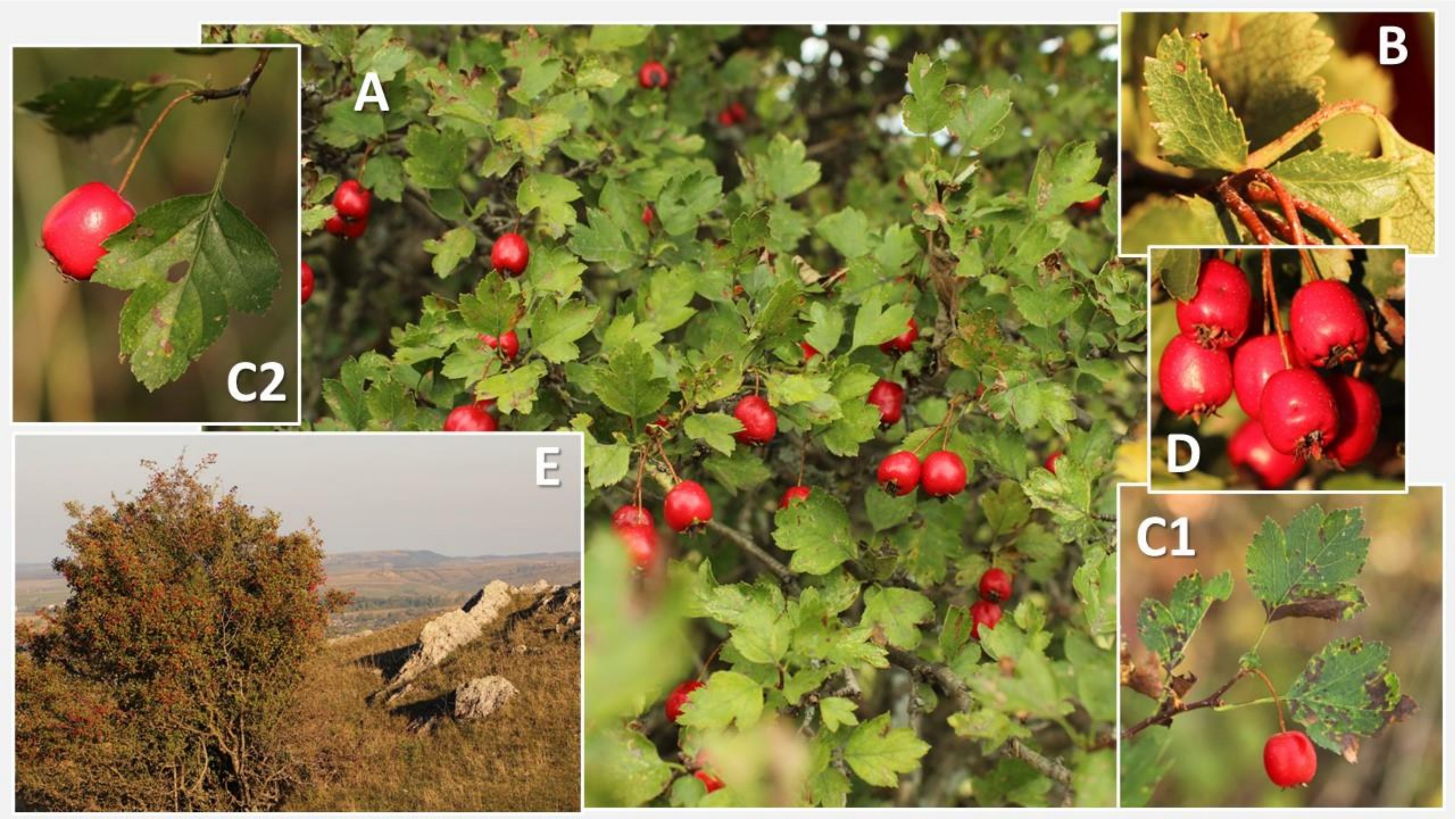


Figure 10 (A-E): *C.* × *macrocarpa* nothovar. *hadensis* (Hrabětová-Uhrova) K.I. Christensen (*C. laevigata* × *C. lindmanii*); **A** – fruiting branches; **B** – stipules of leaves of generative shoots; **C1, C2** – generative shoot with pomes, leaves and their stipules; **D** – pomes with speals and styles; **E** – habitus of a specimen in its natural habitat; (photos: Kuhn Thomas, near Huedin, Romania)

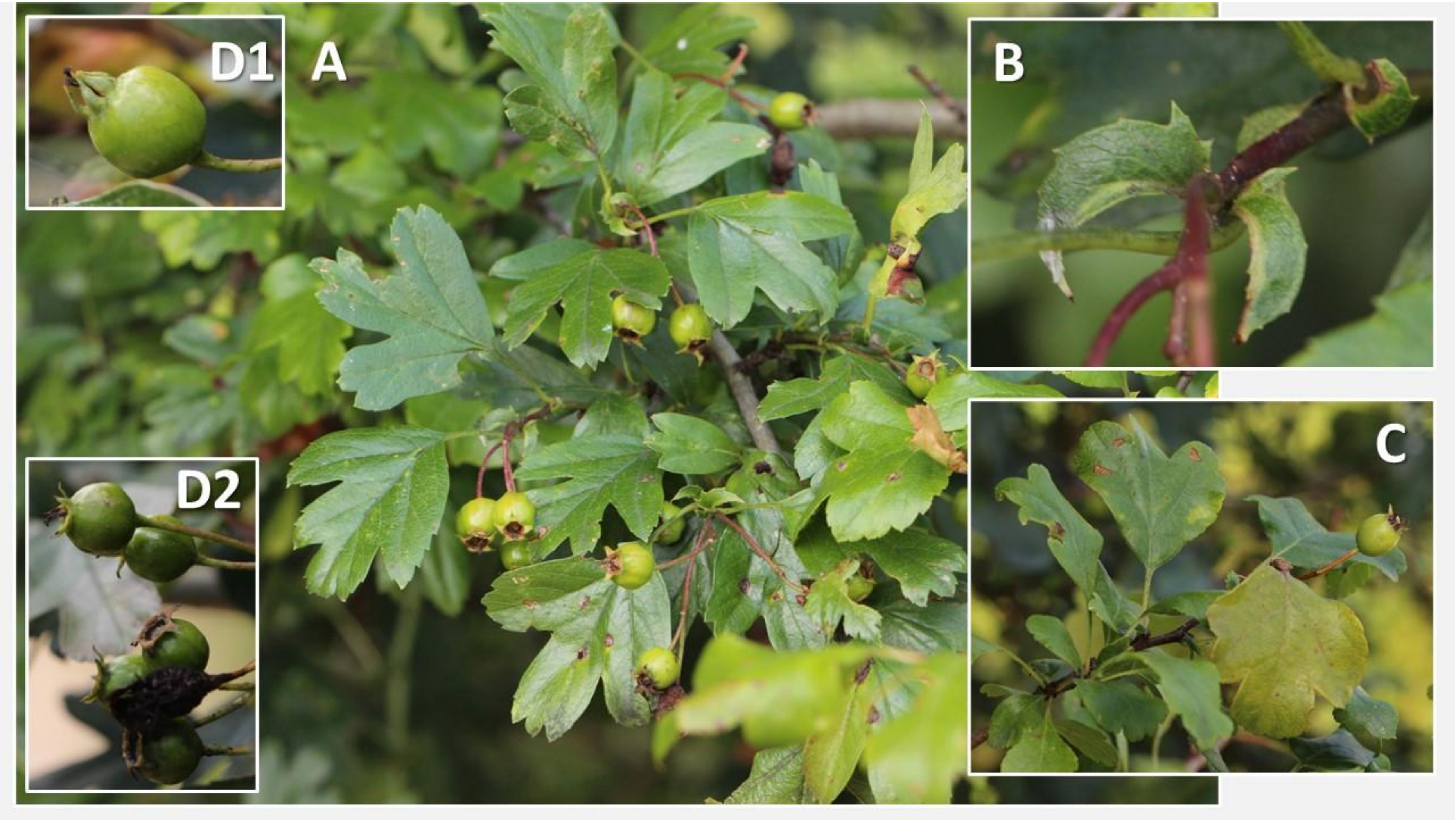
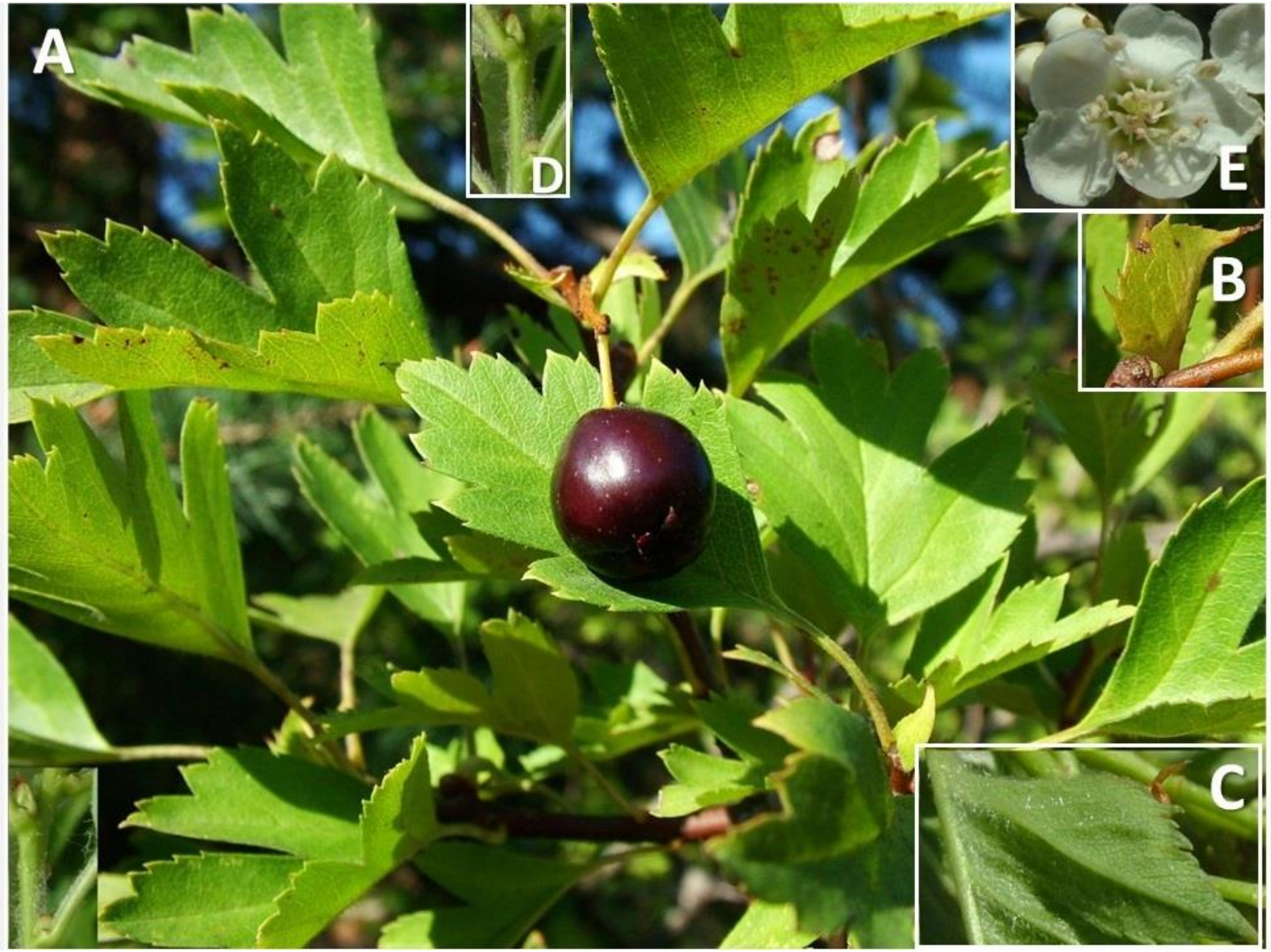


Figure 11 (A – D): Triple hybrid between *Crataegus monogyna* × *C. lindmanii* × *C. laevigata*; **A** – fruiting branches; **B** – stipules of leaves of generative shoot; **C** – generative shoot; **D1, D2** – pomes with sepals and styles; (photos: Kuhn Thomas, near Beiuș, Romania)



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