

POLLINATION ECOLOGY OF *HABERLEA RHODOPENSIS*
FRIV. (GESNERIACEAE), A TERTIARY RELICT ENDEMIC
TO THE BALKAN PENINSULA

Katerina Bogacheva-Milkoteva, Ekaterina Kozuharova,
Regine Claßen-Bockhoff*, Andrej Gogala**

(Submitted by Academician V. Golemansky on May 28, 2013)

Abstract

This study presents preliminary results on the pollination strategy and reproductive success of the tertiary relict and endemic species *Haberlea rhodopensis* Friv., Gesneriaceae in a highly fragmented habitat in Bulgaria and Greece.

The flowers of the species receive very scarce insect visits, mainly from non-specialized insects. The species is obviously not attractive to honeybees and bumblebees and we could not find a specific pollinator. Flowers offer pollen and scarce nectar as a reward. Pollination success is characterized by high seed production and rate of pollination.

Key words: *Haberlea rhodopensis*, pollination, nectar, seed set

The study was conducted with the financial support of a project of Deutsche Bundesstiftung Umwelt, Medical University of Sofia and Johannes Gutenberg University of Mainz, 2011.

Introduction. *Haberlea rhodopensis* is an endemic species of the Balkans. It is a resurrection plant, belonging to the tropical family Gesneriaceae. In Europe there are only three genera of the family: *Haberlea* (in Bulgaria and Greece), *Ramonda* (Balkan Peninsula and Pyrenees) and *Jankaea* (in Greece) [1]. All of them are tertiary relicts, having evolved under climatic conditions, which differ strongly from the recent ones. The flora of Southeastern Europe, the Early to Middle Miocene flora, was rich, diverse in thermophilous elements, consisting of polydominant mesophytic to hygromesophytic forests. Climatic changes after the Miocene climatic optimum caused changes in the floristic composition and vegetation structure, characterized by a decrease in the abundance of palaeotropical and thermophilous elements, a reduction of macrothermic elements, and the disappearance of evergreen laurel forests. Together with these changes, the role of arctotertiary species in plant communities in the mesophytic forests increased. Major vegetation changes occurred in the late Miocene, under more diverse climatic conditions [2].

The flowers of *H. rhodopensis* have a prominent entomophilous syndrome, namely of a “gullet” type with pollen transferred nototribically [3–5] (Figs 1, 2) and our previous studies show that they are incapable of self-pollination. Observations on actual pollination in the Gesneriaceae are scanty [3]. With regard to the European representatives, there are studies on the pollination biology of the related species *Jankaea heldreichii* [6], some observations on honeybee influence on *Haberlea rhodopensis* [7] and on *Ramonda myconi* [8]. Therefore, it is of great interest for the development of conservation strategies for the species to study its pollination characteristics in the context of its relict nature, fragmented habitat and the challenges of the changing environment.

Molecular analysis with nuclear and plastid markers for molecular analysis of five actinomorphic genera in the Old World Gesneriaceae shows that floral actinomorphy in the family has evolved convergently in different alliances. The authors hypothesize that the evolution from zygomorphy to actinomorphy, with novel combinations of characters, is possibly due to shifts in pollination strategies, such as a switch from nectar to pollen rewards and a switch to generalist pollinators. It is suggested that *Haberlea rhodopensis* might be a relict of the stock from which the actinomorphic flowers of *Ramonda* evolved [9].

It has been argued that many of the ways in which humans disturb natural habitats are likely to disrupt plant-pollinator interactions, with consequent out-cross pollen limitation, and that the ecological context of anthropogenic pollen

Fig. 1. Flower visitors of *Haberlea rhodopensis*. Left to right, first row: Pollinating *Lasioglossum morio*. *L. morio* with pollen load. Left to right, second row: Syrphids were frequent flower visitors. Evidence for predation on the flowers →





limitation can generate diverse selective forces on plant mating systems. In many cases, we expect an evolutionary shift towards higher frequencies of self-fertilization, although the selective forces and traits involved are likely to vary with the nature of the disturbance. It will be particularly important to determine whether human disturbance is qualitatively different from natural factors that cause PL and whether plant populations have the demographic and genetic capacity for evolutionary responses adequate to ensure persistence in changing environments [10].

Pollination systems are often more generalized and dynamic than the concept of “pollination syndromes” suggests. Moderate to substantial generalization often occurs. Plant generalization is predicted by a simple model: as long as temporal and spatial variance in pollinator quality is appreciable, different pollinator species do not fluctuate in unison and they are similar in their pollinator effectiveness. In conservation biology, generalized pollination systems imply resilience to linked extinctions, but also the possibility that introduced generalists might displace natives with a net loss of diversity [11].

Flowers in six communities from three continents were scored for expression of floral traits used in published descriptions of the pollination syndromes, and simultaneously the pollinators of as many species as possible were characterized. Ordination of flowers in a multivariate “phenotype space”, defined by the syndromes, showed that almost no plant species fell within the discrete syndrome clusters. Furthermore, in approximately two-thirds of the plant species, the most common pollinator could not be successfully predicted by assuming that each plant species belongs to the syndrome closest to it in the phenotype space. A fresh look at how traits of flowers and pollinators relate to visitation and pollen transfer is recommended [12].

In our study, we compared native populations and plants grown *ex situ* in the Botanical Garden of Mainz University. We tested: 1) the identity of the pollinator agent; 2) the nature of the reward; 3) the extent to which the abundance of the fruit and seed set could be used as estimators of pollination success.

Materials and methods. The study sites. The population at Ustovo district is situated in the Central Rhodope Mts, at 800 m. a.s.l., along Cherna River, on limestone rocks in broadleaf forest with meadows nearby. Co-flowering genera during the observation time in the range of 30 m were: *Taraxacum officinale* L., *Geranium* sp., *Alliaria* sp., *Lamium* sp., *Veronica* sp., *Crataegus monogyna* Jacq., *Syringa vulgaris* L., *Apium* sp., *Rhinantus* sp., *Sambucus nigra* L. The weather during the observation period was mostly cloudy, with rain and storms

← Fig. 2. Flower visitors of *Haberlea rhodopensis* – small size generalist insects

in the afternoon with temperatures at noon between 11.5 °C and 22 °C. In the period between 8.06. and 10.06.2012, the weather was sunny with temperatures 22–30 °C.

The population in the village of Nenkovo is situated in the Eastern Rhodope Mts, at 400 m a.s.l., along Borovitsa River, on limestone rocks in an open area near meadows. Co-flowering genera during the observation time in the range of 30 m were: *Malus* sp., *Prunus* sp., *Salix* sp., *Alnus* sp., *Muscari* sp., *Veronica* sp., *Viola* sp., *Geranium* sp. The weather was sunny, with temperatures of about 18.5 °C.

The cultivated population in the Botanical Garden of Mainz University is in the alpinum of the garden at about 90 m a.s.l. A bumblebee nest was near the stand of *Haberlea rhodopensis*. The weather was rainy, with temperatures of about 14 °C between 26 and 29.04.2012, and in the period 29.04.2012–06.05.2012 there was warm (23 °C) and sunny weather.

Pollinators/visitors. The tests were performed according to standard methods [13]. Most observations were conducted in 2010–2012, during the flowering period from April to July. For in situ observations we chose two natural localities in Central and Eastern Rhodope Mts Bulgaria, and a population from the Botanical Garden at Johannes–Gutenberg University in Mainz, Germany. The total observation time of 52 h was adjusted according to the peak of flowering of the populations and distributed during the periods 16.05.2010–20.05.2010 and 01.06.2012–10.06.2012 at Ustovo district, 24.04.2010–26.04.2010 in the village of Nenkovo and 26.04.2012–06.05.2012 in the Botanical garden of Mainz from 08.00 till 22.00 h. We used the transect method [9]. Previous sporadic observations in four more localities in Rhodope Mts in 2008 and 2009 (villages of Beden, Trigrad and Yagodina) supplied additional information for the study.

Reward – nectar presence and concentration. We obtained preliminary results on nectar presence in the flowers of *H. rhodopensis*, using glucose test strips. In the second phase of the study, nectar concentration was measured at different times of the day on bagged and free flowers with hand refractometer Eclipse 0–50% Sucrose. For estimating nectar quantity, we used glass capillaries of 1 µl, 2 µl, 5 µl and 10µl.

Pollination success. Pollination success was evaluated by: a) free pollination fruit set of a random sample; we collected randomly 36 inflorescences with 177 flowers from four sampling sites at the village of Yagodina and the village of Trigrad in Rhodope Mts; b) seed set per fruit of a random sample on plants of different sampling sites; we counted the seeds, non-developed ovules and damaged seeds from a total of 27 capsules from four different sampling sites. For each sampling site, we calculated the average for each category.

Results and discussion. Pollinating agents. Our observation showed very scarce insect visits, even at the peak of flowering of the population and at a time of high insect activity. Honeybees and bumblebees avoid the flowers of

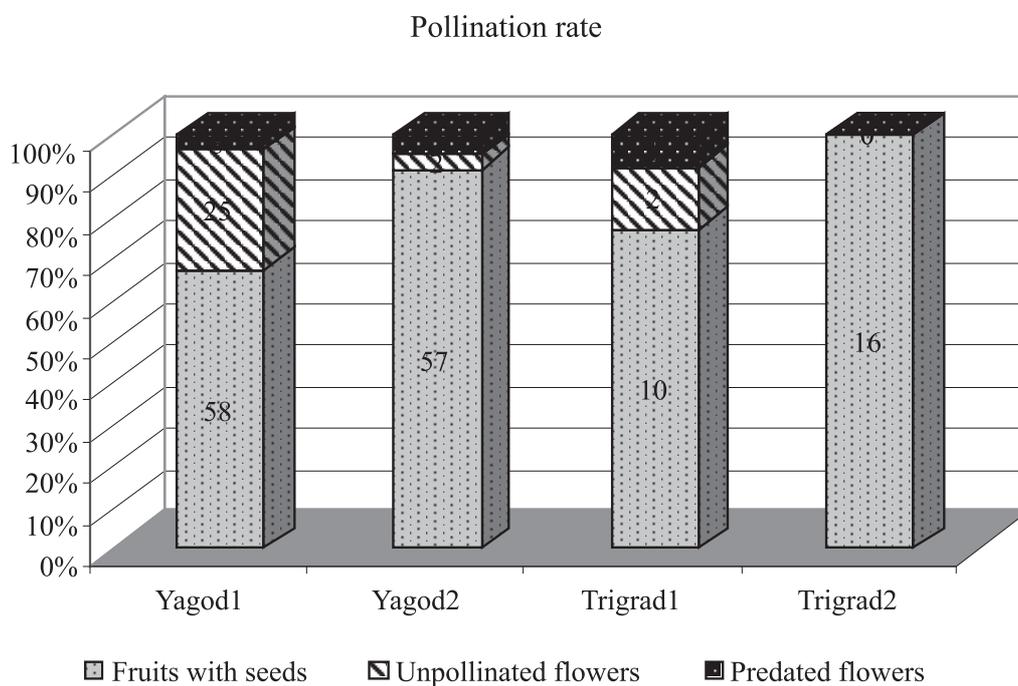


Fig. 3. Pollination rate of a random sample of *H. rhodopensis*

Haberlea rhodopensis and visit other co-flowering species. They rarely approach the flower and do not try to reach pollen or nectar. We compared the populations of *H. rhodopensis* and *Veronica* sp. in Ustovo district, a distance of 1 meter apart, undertaking 40 min observation in conditions of high insect activity. *Veronica* sp. received 18 visits by *Apis mellifera*, while *H. rhodopensis* received 3 visits with duration of no more than two seconds. Many insects approach the flowers without trying to get inside. In the Botanical Garden of Mainz, a bumblebee nest was near the site, but the bumblebees did not visit the flowers of *H. rhodopensis*.

In both natural habitats, we found many small-size visitors, probably using the flowers as shelter or feeding on pollen – ants, caterpillars, beetles, flies (Formicidae, Coleoptera, Diptera, larvae, Fig. 2). We found evidence for predation, pollen or nectar robbing. In the Botanical Garden in warm and dry weather (temperatures above 23 °C), in addition to the above-mentioned, we noted regular pollination visits by *Lasioglossum morio* (Fabricius 1793), collecting pollen, storing it and moving from flower to flower. A group of five *H. rhodopensis* individual flowers received 20 visits in 20 min. Similarly high activity was observed during four days of warm weather between 10 a.m. and 12 at noon. Additionally, syrphids were active flower visitors (Fig. 1).

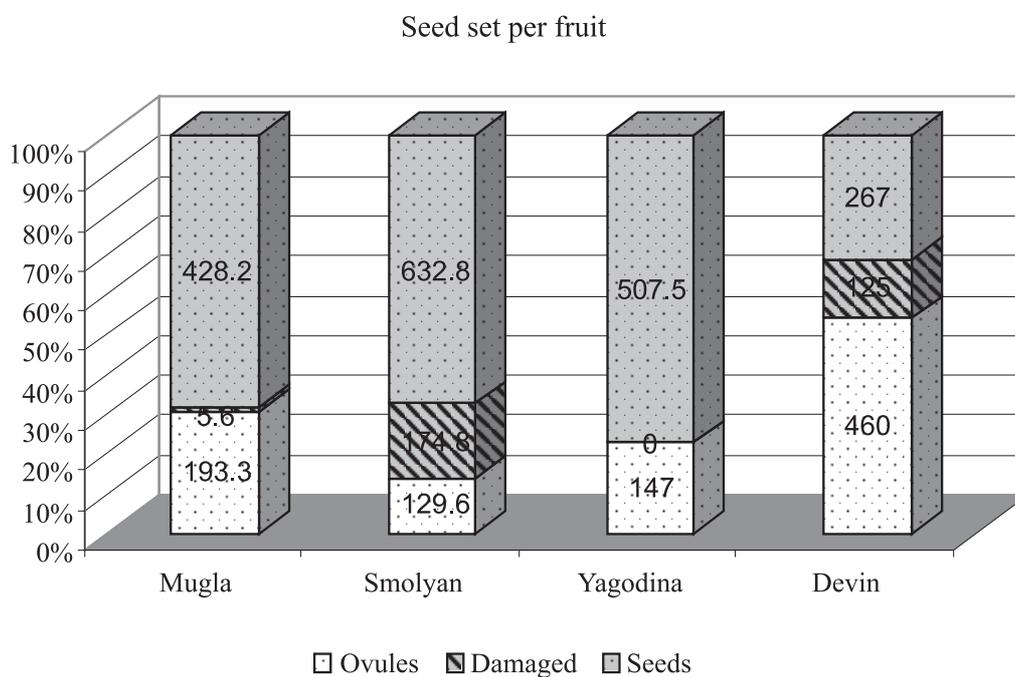


Fig. 4. Seed set per fruit of *H. rhodopensis*

Flowers do not close at night, but by observations after sunset between 6 p.m. and 10 p.m. we could not find any insects at any of the experimental sites.

Despite its typical bee flower characteristics – corolla blue (rarely white) with yellow spots in the mouth, tube widening above, limb bilabiate, nectary annular (Weber) – *Haberlea rhodopensis* is obviously not attractive to bumblebees or honeybees, as our observations in situ revealed. Our previous studies on the pollinators of this plant species show that honeybees do not visit its flowers. The species receives no insect visits except single nectar-collecting *Bombus terrestris* Linnaeus queen and we suggested that pollination is accomplished by nectar-collecting bumblebee queens with long proboscises [7]. This corresponds with studies on the closely related species *Jankaea heldreichii*. The plant is an insect-pollinated generalist without any particular relationship with its insect partners. *J. heldreichii* receives scarce visits only from bumblebee queens – *Bombus lucorum*, *Pyrobombus soroensis* and *P. pratorum* [6]. *Ramonda myconi*, another sister species to *Haberlea rhodopensis*, is mainly visited by bumblebees and syrphids (M. Riba and F.X. Picó, unpublished result). Insects involved in the pollination of the Gesneriaceae include bees (Apiidae, Euglossini in the neotropics, Anthophoridae, Xylocopinae, etc.), rare butterflies and very rare moths. Wasps, dipterans, gnats, etc. are not so far definitely known as pollinators; they probably

play an insignificant role, if any at all [3]. In the recent climatic conditions the plants exist as clone-populations. That is why geitonogamy is probable.

Reward. *Haberlea rhodopensis* has a strikingly orange-coloured nectar disc, but we noted a very low quantity of nectar < 1 µl per flower. Nectar concentration varied between 10 and 42% sucrose, but concentrations above 15% were noted only in sunny weather with temperatures of above 23 °C. We suppose that nectar is produced in the morning, since the highest concentrations are measured between 9 a.m. and 12 at noon. There was no difference between bagged and free flowers. The flowers had a very weak scent.

Flowers of Gesneriaceae are primarily nectar flowers. Lack of nectar (rather rare) is a phylogenetically secondary condition. There are taxa in which the nectary is non-functional or completely reduced – deceptive nectar flowers and pollen flowers. Attraction of pollinators is primarily by visual cues. Most of the flowers are devoid of any floral scent [3]. The genera *Ramonda* and *Jankaia* do not have nectaries [3, 6]. Possibly *Haberlea rhodopensis* is on the way to lose its nectar, due to changes in the pollinator complex. *Haberlea rhodopensis* flowers are self-compatible but do not self-pollinate spontaneously. This plant possesses the ability for vegetative propagation by sending thin, horizontal rhizomes (Bogacheva-Milkoteva, Kozuharova, in press).

Pollination success. From the 177 flowers tested for pollination rate, 141 (79.6%) had fruits, 29 (16.4%) were not pollinated and 7 (4%) were predated.

We recorded an average of 768 ($N_{\text{capsules}} = 27$) ovules per fruit. An average of 458 (59.8%) of them matured to seeds. An average of 232 (30.3%) unset ovules per fruit were counted. An average of 76 (9.9%) predated or damaged ovules per fruit were counted. Generally in Gesneriaceae seeds are produced in great numbers, are small and have little nutrition storage [3]. *R. myconi* fruits contain many small seeds (on average 500 seeds per capsule; M. Riba, unpublished result). In *Jankaia heldreichii*, the number of seeds per capsule varies between 134 and 613. Just 50% of the flowers of *J. heldreichii* survive to fruiting stage [6].

Conclusion. The hypothesis for the pollination syndromes [4, 5] predicts that flowers with a definite complex of flower traits are linked to definite type of pollinators. According to this hypothesis, *Haberlea rhodopensis* flowers should be pollinated by bees. However, we could not observe such a relationship. In situ practically no visitors were observed, except a sporadic one by a nectar-collecting queen *Bombus terrestris* Linnaeus [6]. However, the pollination is effective, if we take into account the results from reproductive success. Ex situ we found only non-specialized visitors and the only bee species we observed was *Lasioglossum morio*, Fig. 1. This social bee is a polylectic [14], widely distributed species [15]. It was a frequent visitor to all flowers in the Botanic Garden.

Our hypothesis. *Haberlea rhodopensis* is a Tertiary relict which, due to its desiccation tolerance and abilities for vegetative propagation, survived dramatic climate changes. During these climatic changes, the local flora changed. This

is probably connected with shifts in the pollinator complexes. In many cases, when there was a specialization to a particular pollinator, because of the climatic changes, the flowers had to switch to more generalized pollination system. Rarely has the path of adaptation to mainly pollen-feeding insects been taken. In the Balkans, Gesneriaceae relicts are such an example of adaptation traits. *Haberlea rhodopensis* has small nectar quantity and lacks specialized visitors in spite of the zygomorphic flower. *Jankaea* could be regarded as the “missing link” in the evolution of the European Gesneriaceae (zygomorphic but without nectary) and *Ramonda* is the next stage – a typical pollen flower.

A similar evolutionary trend is observed in *Dalechampia* (Euphorbiaceae) vines. There is molecular phylogenetic evidence for an evolutionary shift in *Dalechampia* from a highly specialized relationship with resin-collecting bees to generalized pollination by a variety of pollen-feeding insects. This shift was associated with dispersal from Africa to Madagascar, where the specific resin-collecting pollinators are absent. These results show that plants dispersing beyond the range of their specific pollinators may succeed by evolving more generalized pollination systems. Recent fieldwork in Madagascar has shown that the species of *Dalechampia* found there offer only pollen as a reward for pollinators, and that most are pollinated by a variety of pollen-feeding insects, including beetles (Cerambycidae, Scarabidae), muscoid flies (Diptera) and several bees (Halictidae, Anthophoridae, Apidae). It seems that resin-collecting megachilid bees, which are the only pollinators of *Dalechampia* in Africa, failed to colonize Madagascar. The ancestral *Dalechampia* colonists of Madagascar were probably pollinated incidentally by other pollen-feeding insects. They subsequently adapted to the absence of their specific pollinators by losing the gland that secretes the resin reward and by effectively using diverse pollen-feeding insects as pollinators. These changes were sufficiently successful to allow secondary diversification on the isolated island of Madagascar [16]. Historical climate change influences the modularity and nestedness of pollination networks, although this possibility remains poorly tested. This lack of research is in sharp contrast to the considerable efforts to disentangle the role of historical climate change and contemporary climate on species distributions, richness and community composition patterns. On the basis of a global database of pollination networks, it is shown that historical climate change is at least as important as contemporary climate in shaping modularity and nestedness of pollination networks. Specifically, on the mainland is found a relatively strong negative association between Quaternary climate change and modularity, whereas nestedness is most prominent in areas having experienced high Quaternary climate change [17].

What we have to take into account is how viable the seedlings are. Our observations show that in nature very few seedlings survive. Possibly the seedlings cannot compete effectively with the other vegetation. Alternatively, perhaps, they have specialized to mycorrhiza, like the orchids, which are well known for their

symbiosis requirements [18]. The seeds of *Haberlea rhodopensis* are also numerous and tiny. These are implications for our further research.

Jankaea heldreichii occurs only in Mt. Olympus, *Haberlea rhodopensis* reaches further, more to the north in the Rhodope and Stara Planina Mts and *Ramonda serbica*, being least specialised with regard to pollinators, is found on northernmost sites. One possible explanation for the distribution pattern of Gesneriaceae members on the Balkans could be their pollination adaptations, together with other environmental requirements.

Acknowledgements. We express our gratitude to Frank O'Reilly, B.A., Ph.D (Uni. London) for the correction of the English language.

REFERENCES

- [1] GANCHEV I. Rep. Inst. Bot. Bulg. Acad. Sci., **1**, 1950, No 1, 191–214.
- [2] IVANOV D., T. UTESCHER, V. MOSBRUGGER, S. SYABRYAJ, D. DJORDJEVIĆ-MILUTINOVIĆ, S. MOLCHANOFF. Palaeogeography, Palaeoclimatology, Palaeoecology, **304**, 2011, Nos 3–4, 262–275.
- [3] WEBER A. Gesneriaceae. In: The families and genera of vascular plants, Springer, No VII, 2004, 63–158.
- [4] FAEGRI K., L. VAN DER PIJL. The principles of pollination ecology, 3rd rev. edn. Oxford, Pergamon Press, 1979.
- [5] RICHARDS A. J. Plant breeding systems, 2 ed., London, Chapman & Hall. 1997, 529 pp.
- [6] VOKOU D., TH. PETANIDOU, D. BELLOS. Biol. Conserv., **52**, 1990, No 2, 125–133.
- [7] KOZUHAROVA E. Phytol. Balcan., **14**, 2008, No 3, 410–412.
- [8] XAVIER PICO F., M. RIBA. Plant Ecology, **161**, 2002, No 1, 1–13.
- [9] DAPHNI A. Pollination ecology: a practical approach. Oxford Univ. Press, 1992.
- [10] WESTRICH P. Die Wildbienen Baden-Württembergs, 2. verb. Aufl., Ulmer, Stuttgart, 1990, 891.
- [11] EBMER A. W. Linzer biol. Beitr., **20**, 1988, No 1, 527–711.
- [12] WANG Y. Z., R. H. LIANG, B. H. WANG, J. M. LI, Z. J. QUI, Z. Y. LI, A. WEBER. Taxon, **59**, 2010, No 4, 1044–1052.
- [13] ECKERT C., S. KALISZ, M. GEBER, R. SARGENT, E. ELLE, P. O. CHEPTOU, C. GOODWILLIE, M. JOHNSTON, J. KELLY, D. MOELLER, E. PORCHER, R. REE, M. V. MARION, A. WINN. Trends in Ecology and Evolution, **25**, 2010, No 1, 35–43.
- [14] WASER N., L. CHITTKA, M. PRICE, N. WILLIAMS, J. OLLERTON. Ecology, **77**, 1996, No 4, 1043–1060.
- [15] OLLERTON J., R. ALARCON, N. WASER, M. PRICE, S. WATTS, L. CRANMER, A. HINGSTON, C. PETER, J. ROTENBERRY. Annals of Botany, **103**, 2009, No 9, 1471–1480.
- [16] ARMBRUSTER W., B. BALDWIN. Nature **394**, 1998, 632.

- [¹⁷] DALSGAARD B., K. TRØJELSGAARD, A. M. MARTÍN GONZÁLEZ, D. NOGUÉS-BRAVO, J. OLLERTON, T. PETANIDOU, B. SANDEL, M. SCHLEUNING, Z. WANG, C. RAHBK, W. J. SUTHERLAND, J.-C. SVENNING, J. M. OLESEN. *Ecography*, 2013, Early View (Online version of record published before inclusion in an issue).
- [¹⁸] BASKIN C. C., J. M. BASKIN. *Seeds. Ecology, Biogeography and Evolution of Dormancy and Germination*, San Diego, Academic Press, 2001, 890.

*Department of Pharmacognosy
Faculty of Pharmacy
Medical University of Sofia
2, Dunav Str.
1000 Sofia, Bulgaria*

**Johannes Gutenberg-University
Institut für Spezielle Botanik
55099 Mainz, Germany*

***Slovenian Museum of Natural History
Prešernova 20, p.p. 290
SI-1001 Ljubljana, Slovenia*