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REVIEW

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Member of the scientific jury,
according to Order No. 65/04.10.2024 of the Director of IBER-BAS

on Competition for the academic position of "Associate Professor" in professional field 4.3. Biological Sciences, scientific specialty "Botany", for the needs of the Research Group "Resource Assessment and Monitoring of Rare, Medicinal and Aromatic Plants", Section "Applied Botany" of the Department "Plant and Fungal Diversity and Resources" of IBER-BAS, according to the announcement in the State Gazette No. 66/06.08.2024 (p. 121), with the sole candidate Senior Asst. Prof. Dr. Boriana Zdravkova Sidjimova.

General data on the candidate's career and thematic development

Boriana Sidjimova graduated from the Faculty of Botany of Sofia University "St. Kl. Ohridski" in 2002, with a master's degree in Botany, specialization "Medicinal Plants" and in 2001 she started working as a biologist at the Institute of Botany - BAS, and since 2010 at its successor, the Institute of Biodiversity and Ecosystem Research - BAS. In 2008 she acquired the ESD "Doctor" in scientific specialty 01.06.03 "Botany" based on a defended dissertation on the topic: "Biological and phytochemical study of species of the genus *Galanthus* L. (snowdrop) in Bulgaria", and since 2013 she has been a chief assistant in the "Applied Botany" section. In parallel, for 14 years (from 2004 to 2023), she was a part-time assistant professor at the Department of Botany at the Faculty of Biology of Sofia University "St. Kliment Ohridski".

B. Sidjimova's scientific interests are in the field of biodiversity, with a predominance of medicinal plants: resource assessment, conservation and sustainable use, as well as cultivation with the application and evaluation of various methods, incl. hydroponic. She has experience in the monitoring of rare and endangered species, in the management of natural resources, in the development of Management Plans for protected areas and protected zones, as an expert in medicinal plants and in the development of Action Plans for the conservation of plant species. A significant share is occupied by research in the field of phytochemistry and chemotaxonomy, with a pronounced interest in the composition and content of Amaryllidaceae alkaloids in various species of the Amaryllidaceae family and in their biological activity.

Dr. Sidjimova's distinctive features are her high work capacity, responsibility for her

obligations, communication skills, responsiveness, teamwork skills, correctness in relationships and willingness to share her knowledge with colleagues. These qualities define her as a desirable partner in interdisciplinary research and to date she has participated in over 20 scientific projects with national and international funding: from the National Science Foundation, the EEA Financial Mechanism, the Environmental Agency of the Ministry of Environment and Water, etc. She has participated in many national and international botanical conferences, seminars and symposiums. She is a member of the Bulgarian Botanical Society. Her high scientific productivity in recent years is impressive, as for the period from 2021, she has 15 publications in prestigious scientific journals refereed and indexed in the Web of Science and Scopus databases.

Main areas of the candidate's research work and most important scientific contributions in each of them

Sidjimova's research work is entirely related to various medicinal and conservation-significant plant species, mainly from the Bulgarian flora. Her scientific contributions can be grouped into two main areas: 1) Phytochemical studies, related to the study of the composition and content of biologically active substances in medicinal plants (mainly alkaloids) and biological activity of plant extracts; 2) Study of plant diversity and resource potential: ecological features, distribution and monitoring of target species, taxonomic decisions based on anatomical, morphological, embryological and chemotaxonomic studies, studying the influence of increased ploidy on plant growth under controlled conditions.

Contributions to scientific works by indicator B.4. Habilitation work

The publications included in the habilitation report reflect the main direction of the candidate's scientific research: phytochemical and biosystematic study of species of the Amaryllidaceae family: alkaloid composition, chemotaxonomic studies and biological activity. This also includes new data on the distribution of species of the genus Galanthus in Bulgaria and conclusions on their taxonomy based on anatomical, comparative embryological, genetic and morphological studies.

The contributions related to phytochemical studies predominate, published in 7 articles in prestigious scientific journals in the period 2008-2024, proving Sidjimova's enduring interest in Amaryllidaceae alkaloids. Most contributions are on two species of the genus *Galanthus*: *G. elwesii* and *G. nivalis*. In a comparative study of the alkaloid composition of plants of these species, collected from the same habitat, it was found that only 5 out of a total of 37 alkaloids are found in both species. Five new alkaloids have been identified for the genus *Galanthus*. In

addition, the alkaloid composition in individual plant organs has been studied and significant differences have been identified: while in *G. elwesii* homolycorine-type alkaloids predominate, with the exception of the flowers, where they are of the tyramine type, in *G. nivalis* the composition is different in each organ: in the roots lycorine and tazetine-type alkaloids predominate, in the bulbs – tazetine, in the leaves – lycorine, and in the flowers – hemanthamine type. It has been clarified that the biosynthetic pathways of alkaloids in the two species of sympatric populations are different, which is important for their chemotaxonomic differentiation. The research has been deepened by a large-scale study of 25 populations of *G. elwesii* and 7 of *G. nivalis*, in which several chemotypes were established within each species and the geographical distribution of the different chemotypes in Bulgaria was determined. It is also very important to conclude that the alkaloid composition of the two species is genetically determined and remains constant for years during plant cultivation, that is, it can be used as a chemotaxonomic marker. The intraspecific diversity in the alkaloid profile was investigated and a total of 73 alkaloids were identified for the two *Galanthus* species, belonging to 8 main structural types, with 17 alkaloids still unidentified. The studies on Amaryllidaceae alkaloids were expanded to include two more species: *Hippeastrum papilio* (Ravenna) Van Scheepen, which contains galanthamine in a very high concentration, up to 0.54 ± 0.21% of the dry mass; and *Narcissus* cv. *Hawera*, for which seasonal dynamics of accumulation of mesembrine-type alkaloids in different plant organs were established. All the mentioned contributions are original and of potential importance for practice.

Another original contribution is the establishment of inhibitory activity of alkaloid extracts from *G. elwesii* and *G. nivalis* on seed germination and root growth in *Lolium perenne* (English ryegrass). Also of interest is the comparative study of the inhibitory activity of extracts with different compositions.

In an extensive independent study, based on own research and 78 literary sources from the mid-18th century to 2021, information on the distribution of *G. elwesii* and *G. nivalis* in Bulgaria has been systematized and all herbarium specimens have been revised. The horological analysis of the modern distribution of both species shows a wide distribution of *G. elwesii* in the country and limited to *G. nivalis*, as well as some sympatric populations of both species; the ecological characteristics of the habitats of the species of the genus *Galanthus* in Bulgaria have been clarified, and monitoring has shown a good condition of the populations included in the National Biodiversity Monitoring System. To these contributions can be added a contribution in taxonomic terms, based on comparative morphological, comparative anatomical and

embryological studies of the species of the genus *Galanthus* in Bulgaria, showing that the plants identified in some sources as *G. gracilis* are most likely a form of *G. elwesii*.

Contributions to scientific works outside of habilitation work (indicator G.7-8)

Outside the habilitation report, the candidate has presented 16 scientific papers, of which 15 publications are referenced and indexed in the Web of Science and Scopus databases and one chapter in the book Bioprocessing of Plant In Vitro Systems. Reference Series in Phytochemistry, published by Springer. In 2 of the publications, Sidjimova is the first and corresponding author, and in 3 she is the second author; in 2 of the publications she has only one co-author, and in 5 publications she is co-authored with foreign scientists.

The contributions under Indicator G.7-8 are related to different types of research and application of various methods to many types of medicinal plants: phytochemical and anatomical studies, research on biodiversity and resource potential, features of cultivation, as well as the biological activity of extracts. The multifaceted study of the target species is impressive, in which a relationship is sought between structure, ploidy, metabolic profile, biosynthesis of secondary metabolites, biological activity, and also a parallel with the distribution of the species.

Two of the publications (8 and 9) are on the important for the pharmaceutical industry species *Tribulus terrestris*. Thirty two populations have been confirmed in Northern Bulgaria and as a result of resource assessment 19 of them have been determined as economically important. On the other hand, significant differences in the content of important metabolites: three steroidal saponins and the flavonoid glycoside rutin in 16 populations have been established, on the basis of which promising deposits as raw material sources have been identified.

Three of the publications (14, 15 and 22) are dedicated to *Narcissus* cv. *Hawera*, in which 29 alkaloids were identified, of which 13 of the Sceletium type and two new alkaloids; lycorine is the main alkaloid (43.5% of the total alkaloid extract). For the first time, a comparative analysis was carried out between underivatized and silylated alkaloid fractions of the species, as a result of which the sensitivity of the method to compounds with two and more hydroxyl groups was improved. For the first time, Sceletium-type alkaloids were identified in *in vitro* grown plants of *Narcissus pallidulus* and *N. cv. Hawera*. For the first time, it was established that the alkaloid extract of the aerial mass of *Narcissus cv. Hawera* can damage the DNA of *Chlamydomonas reinhardtii*.

Two publications (18 and 21) summarize the research on the galanthamine-rich and poorly studied endangered species *Hippeastrum papilio*. The metabolic profile in the different organs and tissues was established, based on which the assumption was made that the alkaloids are synthesized in the leaves and stored in the bulbs and roots, which have a higher content of galanthamine compared to the leaves. The influence of autotetraploidization on the alkaloid content was studied and it was found that the content of galanthamine and hemantamine in the leaves of tetraploid plants is higher than in diploid ones. In the comparative analysis of diploid and autotertaploid plants, over 60 primary and secondary metabolites were identified and differences in the metabolic profile were proven. It was found that the stomata of autotetraploids are larger than those of diploids, which can be used as a method for rapid screening when obtaining autotetraploids by colchicine. Another characteristic feature of autotetraploid *H. papilio* plants is their delayed development in the first year, observed in hydroponic cultivation.

Based on monitoring of 11 populations of *Colchicum autumnale* in Bulgaria, an assessment of the distribution, status and resource potential of the species was made, as well as a recommendation to collect seeds only from 5 populations characterized by high resource potential (publ. 10). The factors that led to the decrease in population density and the disappearance of some localities were also noted, and anthropogenic pressure was also considered. The population status of the critically endangered species *Rhodiola rosea*, which is listed as critically endangered by the IUCN and protected in Bulgaria by the Biodiversity Act, was also established in 6 localities in Rila Mountain (publ. 13). In connection with the high degree of vulnerability of the species to anthropogenic influence and climate change, the opinion was expressed that additional measures are needed to protect the populations of *R. rosea* and to include the species in the CITES Appendix (Convention on International Trade in Endangered Species of Wild Fauna and Flora).

The ability of essential oils from the species *Monarda fistulosa, Satureja pilosa, Origanum vulgare subsp. hirtum* and *Thymus longedentatus* to inhibit acetylcholinesterase was first established (publ. 16). The anti-acetylcholinesterase activity of these oils was very strong, comparable to that of a methanol extract of *Leucojum aestivum*, which is why it was recommended to investigate their insecticidal activity.

Individual Amaryllidaceae alkaloids, as well as alkaloid extracts and fractions from *Leucojum aestivum* and *Narcissus cv. Hawera*, have been shown to inhibit the growth of endophytic fungi isolated from *L. aestivum*, with the inhibitory effect depending on the fungal

strain (publ. 20). The results provide an opportunity to elucidate the role of Amaryllidaceae alkaloids in endophyte-plant interactions.

Significance of the results obtained

Assist. Prof. Dr. Sidjimova exceeds the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria (LDASRB) and the IBER-BAS for holding the position of "associate professor", with 23 scientific publications other than those related to her dissertation, of which 15 in journals with IF (Web of Science), 7 in journals with SJR (Scopus) and 1 book chapter, distributed in Indicator B (110 points, with a required 100 points) and Indicator G (250 points, with a required 220 points), with 3 publications in journals with quartile Q1, and 4 - in journals with Q2 (publications without quartiles are not presented, since according to the current LDASRB they do not bring her points). Many of the publications are co-authored with foreign scientists. The candidate has presented 42 citations in journals with IF/SJR, for a total of 5 publications, bringing her 84 points (with a total of 20 citations and 60 points required). In fact, the total number of citations without auto-citations is much higher: 188, with 153 citing publications, (H-index 7) according to the information in Web of Science https://www.webofscience.com/wos/author/record/AAE-3556-2019 while according to Scopus https://www.scopus.com/authid/detail.uri?authorId=6504326625 the number of citations without auto-citations is 154 (H-index 6). The candidate did not list all the citations, so for example for publ. No. 3 she presented only 3 citing publications, while in Scopus their number is 7, etc., which explains the higher real H-index. In 4 of the publications Sidjimova is the first author, in 2 publications she is the corresponding author, and in one publication she is the sole author.

Most significant scientific and applied achievements

In both main scientific areas in which Sidjimova works (phytochemistry and biological activity of plant extracts and distribution, monitoring and resource potential of medicinal plants), numerous original contributions are emerging, with the results of the research often opening up opportunities for future application of scientific conclusions in practice.

In the field of phytochemical research, contributions of potential societal importance are related to two plant species containing Amaryllidaceae alkaloids. The high content of galanthamine in *Hippeastrum papilio*, up to 5 times higher than that in the sources currently used for its industrial production, make it a promising competitive species (5). In attempts to cultivate it, *in vitro* propagated plants have been successfully adapted to soil cultivation, and

autotetraploidization has been found to increase the content of galanthamine and hemantamine in the leaves, as well as to cause some differences in the metabolic profile (18). Data on the dynamics of the alkaloid composition in the different organs of *Narcissus cv. Hawera* during the different vegetation phases are important for future pharmacological studies (6).

Studies on the biological activity of secondary metabolites also have potential for practical application. The established inhibitory activity of alkaloid extracts from *Galanthus elwesii* and *G. nivalis*, especially tazetine-type alkaloids, on seed germination and root growth are the basis for the development of new bioherbicidal products (7). The inhibitory effect of distinct fractions of alkaloid extracts from *Leucojum aestivum* and *Narcissus cv. Hawera* on endophytic fungi, as well as the ability of the alkaloid extract from *N. cv. Hawera* to damage the DNA of *Chlamydomonas reinhardtii* could be further studied with a view to application in plant protection (20, 22). The strong antiacetylcholinesterase activity of the essential oils from *Monarda fistulosa, Satureja pilosa, Origanum vulgare subsp. hirtum* and *Thymus longedentatus* is also a prerequisite for their use in the creation of new bioinsecticide preparations (16).

Determining the content of biologically active substances in distinct populations of a given medicinal plant species, on the one hand, and the resource potential of these populations, on the other hand, is very important for making the right decision regarding their use as a raw material for the pharmaceutical industry. As a result of such complex studies, promising localities of *Tribulus terrestris* in Northern Bulgaria have been identified, suitable for exploitation in the production of Tribestan by Sopharma EAD and other pharmaceutical products based on extracts of this species (8, 9). The results of the study on the distribution, state and resource potential of populations of *Colchicum autumnale* are also of practical value due to the application of colchicine as a cell poison, in human and veterinary medicine, in homeopathic medicines, etc. (10).

Critical notes

In presenting her contributions, the candidate made some inaccuracies in the expression, rather inadvertently, e.g. on page 5 “qualitative and quantitative composition of the alkaloid fractions” instead of qualitative composition and quantitative ratio or content; page 10 “strong AChE activity” is actually strong anti-AChE activity. It is recommended that the units of measurement be written as accepted in the SI system (mg, not мг, page 5) and to avoid the use of foreign terms (“review publications”, page 3). Summarizing the information in the scientific literature related to GC-MS analysis is valuable in itself and probably very useful for other

researchers in this field, but in my opinion it cannot be considered a contribution as long as it is not related to the presentation of one's own results (page 9, publ. 23).

In the list of scientific papers, there is a confusion in their numbering before the table: Articles in journals with impact rank – in fact they are Nos. 3, 4, 10, 13, 16, 19, 20, as they are correctly noted in the table itself.

The remarks made in no way diminish the merits of the scientific research carried out and the importance of the results obtained both for the development of science in the indicated areas and for their practical potential.

Skills or aptitude for leading scientific research

Sidjimova has experience in managing work packages on projects funded by the National Science Foundation. Her ability to organize teamwork when conducting scientific research in the phytochemical laboratory and in the greenhouse is impressive. She has also proven her ability to lead a field team for resource assessment and mapping of natural habitats and plant species. These qualities of the candidate have earned the respect and trust of her colleagues, among whom she enjoys authority. Her good communication skills, acquired during the implementation of the projects and during her teaching work at the Faculty of Biology of Sofia University "St. Kliment Ohridski", as well as her active participation in numerous scientific forums, including international ones, show potential for successful management of scientific projects with external funding.

Motivated answer to the question of whether the candidate has a clearly defined research profile

Senior Asst. Prof. Dr. Boryana Sidjimova has 20 years of experience in the specialty, all of which were at the Bulgarian Academy of Sciences: at the Institute of Botany and its successor, the Institute of Biodiversity and Ecosystem Research. The profile of her research work is clearly outlined, related to the study of various types of medicinal plants and *in situ* and *ex situ* activities for the protection of conservation-significant plant species. Her activity in all scientific projects in which she has participated to date is related to phytochemical studies of medicinal plants, research of the biological activity of plant extracts, monitoring and resource assessment of species of economic importance, monitoring of conservation-significant species and biodiversity conservation.

Role of the candidate in the training of young scientists

Sidjimova has many years of teaching experience as a part-time assistant professor in the Botany Department of the Faculty of Biology at Sofia University, with a total of 1932 hours since 2004, of which 1620 hours of lectures in "Systematics of Vascular Plants", "Botany II" and "Biodiversity of Plants and Fungi" and 312 hours of summer internship in "Botany II" and "Biodiversity". She also has one student from the Faculty of Biology at Sofia University who successfully defended her master thesis in 2023.

A reasoned conclusion recommending unequivocally the selection or not of the candidate

Senior Asst. Prof. Dr. Boriana Sidjimova meets all the requirements for the position of “Associate Professor” in the field 4.3. “Biological Sciences”, according to the Regulations for the Implementation of the Law on the Development of the Academic Staff in the Republic of Bulgaria, as well as the criteria of IBER. She is a well-established scientist, with extensive knowledge in the field of medicinal plants, high work capacity, skills to apply various research methods and interpret the results obtained. She is distinguished by correctness in collegial relationships, organizational skills, motivation to train students and younger colleagues and has the potential to lead scientific projects.

In conclusion, I strongly recommend that the esteemed scientific jury evaluate the activities of Senior Assistant Professor Dr. Boriana Sidjimova and vote for her election to the academic position of "Associate Professor" in professional field 4.3 "Biological Sciences", scientific specialty "Botany".

22.11.2024 г. Prof. M. Stanilova, PhD