Arctoa (2022) **31**: *52–58*
doi: 10.15298/arctoa.31.07

NEW DATA ON DISTRIBUTION, PHYLOGENETIC AFFINITY AND SPOROPHYTE OF *NARDIA PACIFICA* BAKALIN

НОВЫЕ ДАННЫЕ О РАСПРОСТРАНЕНИИ, ФИЛОГЕНЕТИЧЕСКОМ ПОЛОЖЕНИИ И СПОРОФИТЕ *NARDIA PACIFICA* BAKALIN

NADEZHDA A. KONSTANTINOVA[1] & ANNA A. VILNET[1]

НАДЕЖДА А. КОНСТАНТИНОВА[1], АННА А. ВИЛЬНЕТ[1]

Abstract

The liverwort *Nardia pacifica* Bakalin recently described from Kamchatka is recorded for the Caucasus and Alaska. Molecular phylogenetic estimation based on two newly sequenced and deposited in GenBank chloroplast DNA markers – *trn*L-F and *trn*G-intron – of the firstly studied type specimen of this species and specimens from these two remote areas suggested their high similarity. The description and microphotographs of male and female plants as well as sporophyte are provided here for the first time. Morphological and molecular infraspecific variabilities are discussed.

Резюме

Недавно описанный с Камчатки печеночник *Nardia pacifica* Bakalin выявлен на Кавказе и Аляске. Филогенетический анализ рода показал почти полную идентичность ДНК последовательностей хлоропластных маркеров *trn*L-F и *trn*G-интрона изотипа и образцов с Аляски и Кавказа, впервые полученных и депонированных в ГенБанк. Впервые для вида приводится описание и фотографии мужских и женских растений, а также спорофита. Рассматривается морфологическая и генетическая вариабельность вида.

KEYWORDS: *Nardia,* Gymnomitriaceae, molecular phylogeny, morphological variability

INTRODUCTION

In 2009, on Mount Aibga (Caucasus, Krasnodar Territory of Russia), the senior author collected a specimen of *Nardia*, which could not be attributed to any of the known species of the genus. The specimen was studied when fresh and the oil bodies were described as homogeneous, which did not allow us to attribute it to *N. japonica* Steph., while the leaves, which were relatively deeply divided into two lobes, made it impossible to determine it as *N. scalaris* Gray, a species which has homogeneous oil bodies. The specimen marked as *Nardia* sp. nova was laid aside and forgotten. However, the publication of *Nardia pacifica* Bakalin (Bakalin & Klimova, 2016) from the Kamchatka Territory of Russia reminded us of this finding. A re-examination of the morphology of the specimen showed that it clearly corresponds to the description of *N. pacifica*. In addition, during the recent study of the collection from Alaska, the first author identified several specimens as *N. pacifica*. Despite the fact that the specimens were collected in Alaska almost 30 years ago, we were able to isolate DNA from them as well as from a Caucasian specimen to check if they belong to a single species. Of great interest is the fact that female plants, including mature sporophytes, were found in the specimens from Alaska, whereas male plants were represented in the specimen from Caucasus which made it possible to describe gametangia and sporophytes for the first time. The data obtained significantly supplement the original description of the species and expand the understanding of its ecology, distribution range, morphological variability and phylogenetic affinity.

MATERIAL AND METHODS

Sampling

Morphological investigation is based on specimens collected by the senior author in the Caucasus and Alaska and the isotype of *Nardia pacifica* preserved in KPABG. All specimens were examined and photographed by light microscopy using a Leitz Wetzlar Orthoplan microscope equipped with a Nikon D90 digital camera.

Specimen examined: RUSSIA: Krasnodar Territory, Caucasus Mountains, Aibga Mountain Circus facing north near the ski lift, 43.630375°N, 40.294131°E, 2195 m alt., on soil near trail in snow bed meadows with dominance of low herbs and *Sibbaldia*, coll. N.A. Konstantinova & A.N. Savchenko, K209-1-09, 9 Oct 2009 [KPABG 125158]; Kamchatka Territory, East Kamchatka, upper course of Nalycheva River, near Pinachevsky Pass (53°26'31"N, 158°39'08"E), 900 m alt., alpine belt, moist boulder near stream, coll. V.A. Bakalin, K-67-7-15, 19 Aug 2015 [KPABG 120483] (isotype); U.S.A.: Alaska, Matanuska-Susitna Borough, Talkeetna Mtns, Hatcher Pass, siliceous outcrops on tundra slopes with E aspect, 61.76667°N, 149.3°W, 1200 m. alt., on frost boil in late

[1] – Polar-Alpine Botanical Garden, Kola Sci. Centre, Russ. Acad. Sci., Kirovsk-6, Murmansk Province, 184256 Russia; e-mail: nadya50@list.ru; ORCID (NK): 0000-0002-7600-051; (AV): 0000-0001-7779-2593

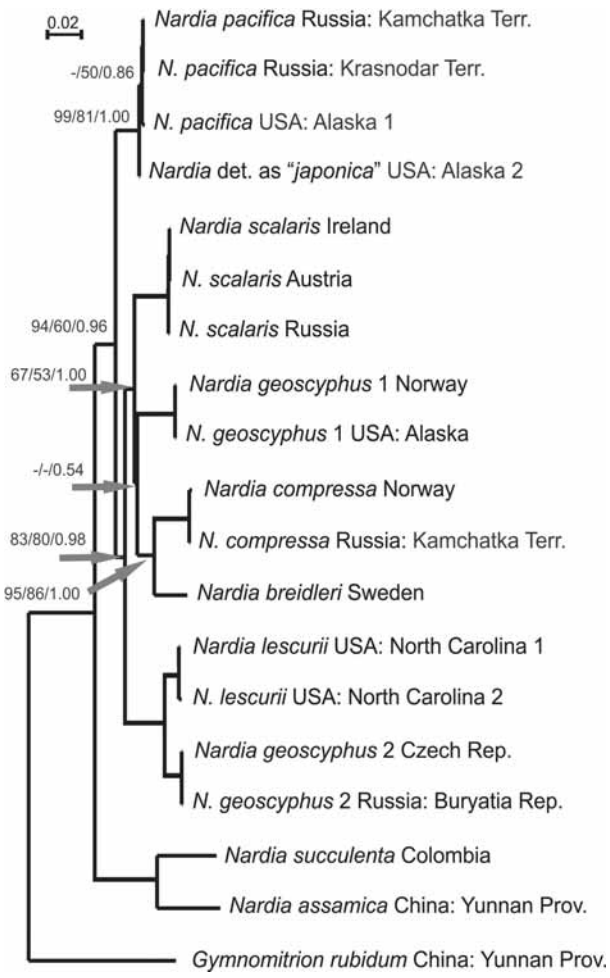

Fig. 1. Majority rule consensus tree from Bayesian estimation based on combined dataset of *trn*L–F + *trn*G-intron cpDNA for the genus *Nardia*. The BS values from MP and ML analyses and PP from BA are indicated, branches to nodes obtained the highest support (100/100/1.00) are in bold.

snow bed, dominated in mats with admixture of *Solenostoma sphaerocarpum, Neoorthocaulis floerkei, Anthelia juratzkana, Pleurocladula albescens*, coll. N.A. Konstantinova, K122-92, 07.VII.1992 [KPABG 123875]; ibid., on edge of frost boil on steep slope, on peat soil, dominated in mats with admixture of *Barbilophozia sudetica, Pleurocladula albescens, Anthelia juratzkana*, coll. N.A. Konstantinova, K131-2-92, 07.VII.1992 [KPABG 123884].

For molecular estimation of variability and phylogenetic affinity of *Nardia pacifica* the isotype from Kamchatka Territory and the specimens from Krasnodar Territory and Alaska were selected. The nucleotide sequence data of *trn*L-F and *trn*G-intron cpDNA of 32 accessions corresponding to 15 *Nardia* specimens and *Gymnomitrion rubidum* as an outgroup taxon were downloaded from GenBank. The list of tested specimens with voucher details and GenBank accession numbers is shown in Table 1.

DNA isolation, PCR amplification and DNA sequencing

DNA from dried plants was extracted with DNeasy Plant Mini Kit (Qiagen, Germany) followed by manufacture's instructions. We amplified and sequenced two DNA loci – *trn*L-F with primers suggested by Taberlet *et al.* (1991) and *trn*G-intron with primers from Shaw *et al.* (2005). PCR was carried out in 20 µl volumes with the following amplification cycles: 3 min at 94°C, 30 cycles (30 s 94°C, 40 s 56°C (*trn*L-F) or 64°C (for *trn*G-intron), 60 s 72°C) and 2 min of final extension time at 72°C. Amplified fragments were visualized on 1% agarose TAE gels by EthBr staining, purified with Cleanup Mini Kit (Evrogen, Russia), and then were sequenced with the ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, USA) following the standard protocol provided for 3100 Avant Genetic Analyzer (Applied Biosystems, USA).

Phylogenetic analyses

The obtained sequences were assembled and then aligned with downloaded accessions in two – *trn*L-F and *trn*G-intron – datasets in BioEdit 7.0.1 (Hall, 1999); missing parts of sequences and unsequenced loci were coded as missing. Preliminary phylogenetic estimation suggested congruent topologies and both datasets were combined into a single one. Phylogenetic analyses provided with the maximum parsimony method (MP) (TNT 1.5, Goloboff & Catalano, 2016), the maximum likelihood method (ML) (Mega 11, Tamura *et al*., 2021) and Bayesian reconstruction (BA) (MrBayes v. 3.2.1, Ronquist *et al.*, 2012). The MP analysis was run with New Technology Search with a search for the minimum-length tree by five reiterations and 1000 bootstrap resamplings. The model T93+I+Γ was chosen as the best-fit evolutionary model of nucleotide substitutions in Mega 11 and then used in ML estimation with four rate categories of gamma distribution and bootstrap resampling procedure with 500 replicates. In the BA *trn*L-F and *trn*G-intron partitions were separately assigned the GTR+I+Γ model as recommended by the program's creators. Two independent runs of the Metropolis-coupled MCMC were used to sample parameter values in proportion to their posterior probability. Each run included three heated chains and one unheated, and two starting trees were chosen randomly. Chains were run for one million generations and trees were sampled every 100th generation. The software tool Tracer (Rambaut *et al.*, 2018) revealed the effective sample size as 7247.0752, auto-correlation time as 248.3788. The first 25% trees were discarded as burn-in in each run. Thereafter 15000 trees were sampled from both runs. The average standard deviation of split frequencies between two runs was 0.001875. Bayesian posterior probabilities were calculated from trees sampled after burn-in procedure. Majority rule (MJ) consensus tree was calculated after combining the runs minus burn-in of 25% and the topology was illustrated with FigTree v. 1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/).

Table 1. The list of specimens tested in phylogenetic estimations, accessions obtained in current study marked by bold, others were downloaded from GenBank.

Taxon	Specimen voucher	GenBank accession number	
		*trn*L-F cpDNA	*trn*G-intron cpDNA
Gymnomitrion rubidum (Mitt.) Váňa, Crand.-Stotl. & Stotler	China: Yunnan Prov., Long 34462 (DUKE)	KF943103	KF942938
Nardia assamica (Mitt.) Amakawa	China: Yunnan Prov., B. Shaw, 5663 (DUKE)	KF943017	KF942878
Nardia breidleri (Limpr.) Lindb.	Sweden, J. Váňa 15.07.2003 (F)	KF942995	KF942860
Nardia compressa (Hook.) Gray	Norway, P. Sova s.n. (DUKE)	KF943076	KF942913
N. compressa	Russia, V. Bakalin, K-74-9-04 (F), 106798 (KPABG)	KF942978	KF942847
Nardia geoscyphus (De Not.) Lindb. 1	Norway, P. Sova s.n. (DUKE)	KF943066	KF942905
N. geoscyphus 1	USA: Alaska, B. Shaw 7210 (DUKE)	KF943012	KF942874
N. geoscyphus 2	Czech Republic, P. Sova s.n. (DUKE)	KF943067	KF942906
N. geoscyphus 2	Russia: Buryatia Rep., N.A. Konstantinova 148-01 (F), 104392 (KPABG)	KF942976	KF942845
Nardia lescurii (Austin) Underw.	USA: North Carolina, 1, B. Shaw, 12987 (DUKE)	KF943013	KF942875
N. lescurii	USA: North Carolina, 2, B. Shaw, 7209 (DUKE)	KF943048	KF942887
Nardia pacifica Bakalin	Russia: Kamchatka Terr., V. Bakalin, K-67-7-15 (VBGI), 120483 (KPABG), isotype	**OK562108**	**OK562111**
N. pacifica	Russia: Krasnodar Terr., N. Konstantinova & Savchenko, K209-1-09, 125158 (KPABG)	**OK562107**	**OK562110**
N. pacifica	USA: Alaska, 1, N. Konstantinova, K131-2-92, 123884 (KPABG)	**OK562109**	no data
N. pacifica	USA: Alaska, 2, W.B. Schofield (DUKE), published as *N. japonica* Steph. (Shaw et al., 2015)	KF943104	KF942939
Nardia scalaris Gray	Austria, P. Sova s.n. (DUKE)	KF943074	KF942911
N. scalaris	Ireland, D. Long 35628 (E)	KF942948	KF942819
N. scalaris	Russia, N.A. Konstantinova & A.N. Savchenko 1/3/2002 (F)	KJ802077	KJ802049
Nardia succulenta (A.Rich. ex Lehm.) Spruce	Colombia, J. Benavides s.n. (SIU)	KF943094	KF942927

The *trn*L-F and *trn*G-intron nucleotide sequence variability was estimated as *p*-distances in Mega 11 using the pairwise deletion option for counting gaps.

RESULTS

The nucleotide sequences were obtained for *trn*L-F of all three specimens and *trn*G-intron - for specimens from Kamchatka and Krasnodar Territories. Five newly generated accessions were deposited in GenBank (Table 1). The *trn*L-F+*trn*G-intron dataset consists of 1219 positions, among them 495 positions belong to *trn*L-F and 724 to *trn*G-intron. The number of invariable sites in the *trn*L–F and *trn*G-intron are 397 (80.20%) and 528 (72.92%) respectively, variable positions are 86 (17.37%) and 181 (25.00%), parsimony informative positions are 54 (10.91%) and 100 (13.81%).

Six equally parsimonious trees with a length of 503 steps were revealed in MP, with CI = 0.823691 and RI = 0.836317. A single tree with the arithmetic mean of Log likelihood -3487.47 was produced in ML. In Bayesian analysis arithmetic means of Log likelihoods for each sampling run were -3451.45 and -3451.08. All obtained topologies became quite similar, thus Fig. 1 demonstrates the MJ consensus tree achieved in BA estimation with indication of bootstrap support (BS) values from MP and ML analyses and Bayesian posterior probabilities (PP) from BA.

Three *Nardia pacifica* specimens sequenced here located in the terminal clade with a specimen from Alaska (W.B. Schofield, DUKE) previously published as *Nardia japonica* (Shaw *et al*., 2015) with the following support values: BS =99% in MP, BS =81% in ML and PP=1.00 in BA. The divergence of *Nardia pacifica*-clade from the remaining *Nardia* species obtained supports BS=94% in MP and PP=0.96 in BA. Multiple *Nardia compressa* and *N. scalaris* also formed their own clades with 100% supports in all estimations. Two specimens of *Nardia geoscyphus* from Norway and USA (marked "1") formed their own clade in unsupported affinity (PP=0.54 in BA) to the clade of *N. compressa* + *N. breidleri* (BS =95% in MP, BS =86%in ML and PP=1.00 in BA). The subclade with two specimens of *Nardia geoscyphus* from the Czech Republic and Russia (marked "2") located in sister relation to the subclade with two American specimen of *Nardia lescurii* with the highest support. *Nardia succulenta* and *N. assamica* are sister related (the highest support in all estimates) and placed in the tree base.

The *p*-distance calculation resulted in the identity of the *trn*G-intron among all tested specimens of *Nardia pacifica*-clade and slight difference in *trn*L-F (0.2%) (Table 2). The difference in *trn*L-F is found for specimen Alaska2 whereas specimen Alaska1 is identical to both Russian specimens in this locus. The divergence of *Nardia pacifica* from the other species of the genus varies

Table 2. The infraspecific and infrageneric DNA sequences variability in the genus *Nardia*, *p*-distances, %.

Taxon	Infraspecific *p*-distances, %, *trn*L-F/ *trn*G-intron	Infrageneric *p*-distances, %, *trn*L-F/*trn*G-intron							
		N. pacifica	*N. scalaris*	*N. geoscyphus 1*	*N. compressa*	*N. breidleri*	*N. lescurii*	*N. geoscyphus 2*	*N. succulenta*
N. pacifica	0.1/0.0								
N. scalaris	0.0/0.0	3.8/3.9							
N. geoscyphus 1	0.0/0.0	4.4/4.0	3.7/3.6						
N. compressa	0.0/0.1	3.8/4.9	2.9/5.4	4.0/5.1					
N. breidleri	n/c/ n/c	4.1/4.6	2.6/4.9	4.6/4.7	2.6/4.2				
N. lescurii	0.2/0.0	4.5/4.2	3.6/4.5	3.8/4.9	3.9/6.7	4.5/6.0			
N. geoscyphus 2	0.0/0.0	4.6/4.3	4.1/5.1	3.9/5.4	4.5/6.5	5.1/6.6	1.2/1.8		
N. succulenta	n/c/ n/c	7.0/7.9	7.2/9.3	7.5/8.7	7.3/9.6	8.0/10.0	7.8/9.5	8.1/9.7	
N. assamica	n/c/ n/c	6.4/7.6	6.7/9.7	7.4/9.1	6.7/10.5	7.2/10.8	7.8/9.5	8.1/9.9	4.1/6.7

from 3.8% till 7.0% in *trn*L-F and from 3.9% till 7.9% in *trn*G-intron. The other *Nardia* species were also characterized by absent or slight nucleotide sequence variability: 0- 0.2% in *trn*L-F and 0-0.1% in *trn*G-intron. The lowest level of nucleotide sequence divergence was detected between *Nardia geoscyphus* "2"and *Nardia lescurii*: 1.2% in *trn*L-F and 1.8% in *trn*G-intron. The other species are more robustly separated from each other: 2.6-8.1% in *trn*L-F and 3.6-10.8% in *trn*G-intron.

The phylogenetic separation from the known species and molecular similarity among samples from geographically remote regions including isotype specimen suggested *Nardia pacifica* as a robust species.

Taxonomy

Bakalin (Bakalin & Klimova, 2016) described *Nardia pacifica* only based on vegetative plants. We found male plants in the specimen from the Caucasus and both male and female plants as well as sporophytes in the specimens from Alaska. It allowed us to describe these structures. The study of specimens from the Caucasus and Alaska revealed some variability of vegetative plants which complements the first description. Below we provide the description of the species based on the specimens we studied.

Nardia pacifica Bakalin, Bot. Pacif. 5(2): 45, 1, 2.2016. Fig.2.

Shoots are 0.9–1.2 mm wide and to 12 mm long; stem straight to slightly flexuose, with both lateral and ventral intercalar branches and intercalar stolones and small-leaved branches; rhizoids sparse, mostly at base of plant or numerous at base of perigynium. Stem cross-section wide elliptical, 200–210×230–240 μm, cells of cross-section not differentiated, thin-walled, with small but distinct trigones, mostly large, 20–25×25–35 μm, with admixture of small cells just 12–15 μm as wide as long. Leaves obliquely inserted, plane or slightly concave, light green to light warm brown (in Alaska) and rather pellucid at base and in the middle but with upper parts along margins sometimes brownish to red brownish, especially in the upper parts of shoots, suborbicular to obliquely trapezoidal, widest below the middle, 600–700 μm wide and 500–650 μm long, divided to 0.2–025(–0.3) of its length by obtuse, crescending to V-shaped sinus, lobes uneven, mostly obtuse, subacute or rounded. Underleaves distinct, large, lanceolate, 100–150×200–350 μm, spreading, triangular, with apex almost straight. Cells thin-walled, subisodiametric, with distinct, relatively large trigones, (17–)19–22(–25)×(20–)22–28(–30) μm in the middle of leaves and 25–30×30–45 μm near the base, where cells often are bistratose in 2–3 rows. Oil-bodies long persistent, homogenous, glistening, 2–3 per cell, rounded, 5–6 μm in diameter or ellipsoidal, 5×9 μm.

Dioicous. Androecium consists of 3–5 pairs of bracts that are slightly larger, wider and more concave than leaves, ca. 700–800 μm wide and 500–550 μm high, with two antheridia that have two-seriate stalks.

Female bracts lager than leaves, 900–1000 μm wide and 800 long, 3 pairs on large fleshy stem perigynium that is ca. 1000 μm long and ca. 600 μm wide, with numerous rhizoids on ventral side, especially at base. Perianth very short, completely hidden among the bracts placed at the tip of perigynium, mouth of perianth crenulate, consists of elongated, slightly protruding and expanding to the upper end of cells ca. 6–10×(13–)15–29 (–22) μm.

Capsule dark brown, ovoid-cylindrical, 450 × 600 μm, capsule walls 2 layers, epidermal cells with nodular thickenings ca. 1.5 as thick as inner walls, with 5–7 annular to semiannular bands. Elaters 8–10 μm wide, with bands 2.5–3.5 μm wide. Spores brown, finely papillate, 12–14 μm (Fig. 2)

Variation and differentiation. In general both Caucasian and Alaskan plants fit well to the description of the species. Plants from the Caucasus differ slightly in presence of intercalary ventral stolones and small-leaved shoots, which is not mentioned by the authors of the species description. In addition, Caucasian plants in general have a slightly smaller sinus and smaller midleaf cells,

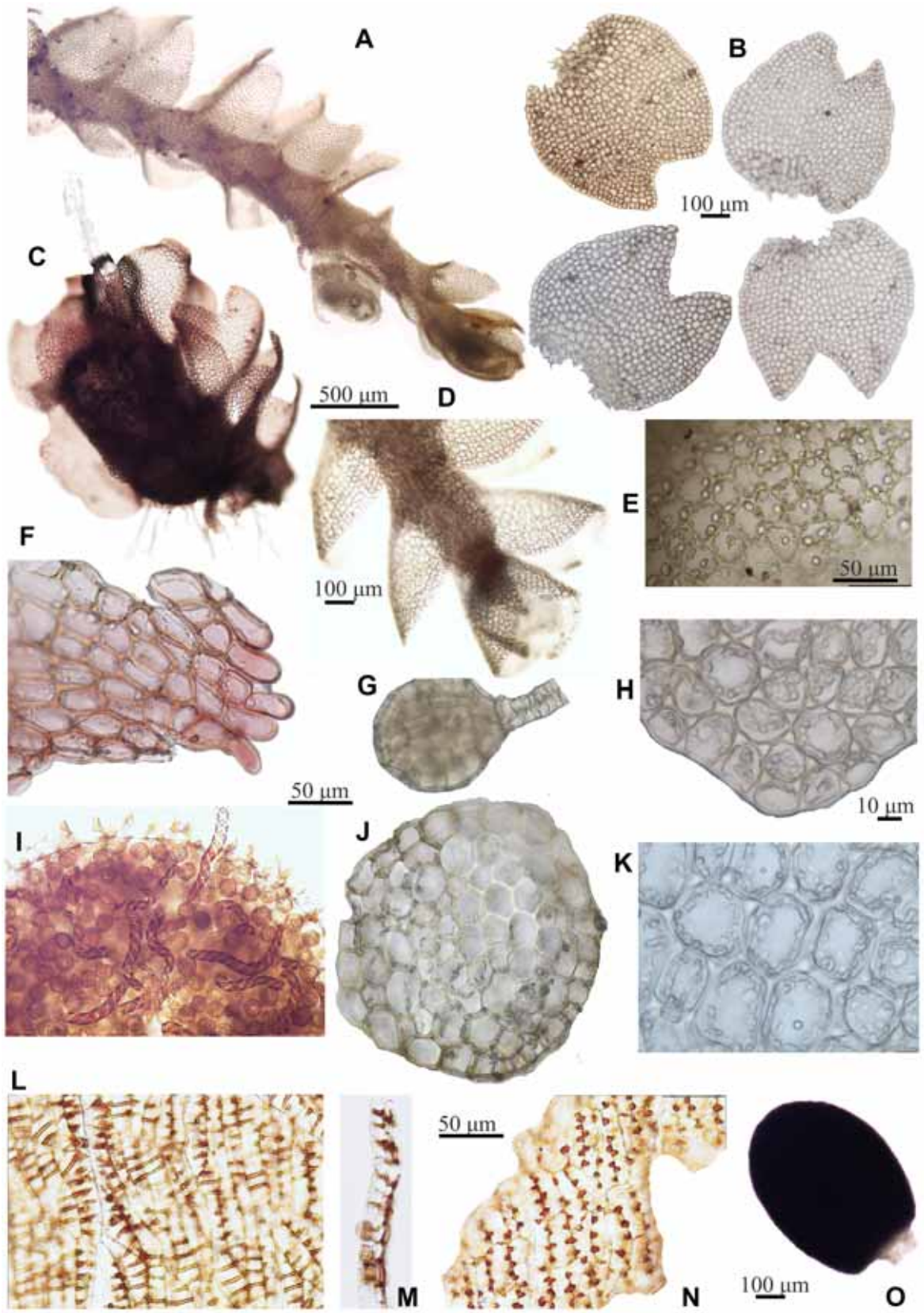

Fig.2. *Nardia pacifica* (A, B, C, D, E, F, G,-H, K, L from K209-1-09; I, M, N, O, P from K131-2-92, KPABG). A: ventral side of shoot; B: leaves; C: perigynium; D: ventral side with underleaves; E: midleaf cells with oil bodies; F: cells of perianth mouth; G: antheridium; H: cells of leaf lobe; I: cross section of capsule filled with spores and elaters; J: cross section of stem; K: cells of base of leaf; L: inner cells of capsule wall; M: cross-section of capsule wall; N: epidermal cells of capsule wall with nodular thickenings: O: capsule.

the values of which are closer to the lower limit described for the type. Plants from Alaska are slightly smaller in size, with a shoot width no more than 1 mm and are mostly more deeply brown colored with margins of leaves red-brown in upper leaves in some plants.

The differences between *Nardia pacifica, N. japonica* and *N. lescurii* are described in detail by Bakalin & Klimova (2016) and briefly consist in the smaller size of *N. japonica* and the larger size of *N. lescurii* compared to *N. pacifica,* as well as the structure and size of the oil bodies that are smooth in *N. pacifica* but coarsely granulate in *N. japonica* and more numerous and finely granulate papillose in *N. lescurii*. From *Barbilophozia sudetica* occurring mixed with *Nardia pacifica* in Caucasus *Nardia pacifica* differs in smaller size, color and presence of distinct large underleaves.

Ecology. Both in the Caucasus and Alaska, the species was collected on moist sandy soil in late snow bed, mixed with arctomontane species typical for such habitats, e.g. *Anthelia juratzkana*, *Barbilophozia sudetica, Pleurocladula albescens.* In both specimens the species is scattered among other bryophytes. The type specimen was collected on moist boulder in alpine belt. Apparently, we can characterize the species as a hygromesophyte.

Distribution. Currently, the species is reliably known from the Caucasus, the Far East of Russia and Alaska. Bakalin & Klimova (2016) presumably referred to *Nardia pacifica* specimens from Karelia Republic (European part of Russia) and Washington State (U.S.A.) which they studied without oil-bodies and also suggested that the records of *Nardia japonica* for Timan, Komi Republic of Russia (Schljakov, 1981) and Alaska (Hong & Váňa, 2000) could also be attributed to *Nardia pacifica*. Our data confirm the wide distribution of the species, and taking into account the discovery the species in the Caucasus, even show a much wider distribution of the species than was supposed by the authors of its description (Bakalin & Klimova, 2016). Based on these data we can state that the species has an arctomontane suboceanic, almost circumpolar distribution.

Discussion and Conclusion

Bakalin & Klimova (2016) clarified the morphological concept of *N. japonica* including a critical revision of different treatments of the species and described *N. pacifica* based on morphological differences of this species from *N. japonica*. Considering, however, that one of the main differences between *N. japonica* and *N. pacifica* lies in features of the oil bodies, which have not been preserved in the type as well as in a majority of herbarium specimens, the molecular genetic study of *N. japonica* specimens from the type or close to its locality is of particular importance. In this work, we were able to sequence DNA and identify molecular similarities in the isotype and two more samples of *N. pacifica*, however an obvious disadvantage of both Bakalin & Klimova (2016) and our studies is the lack of sequenced specimens of *N. japonica.* Unfortunately, we could not find a suitable material of this species for sequencing. Therefore, until it is possible to study live material and obtain sequences of *N. japonica* from the type or close to the type locality, all conclusions on taxonomical status and volume of the latter species are preliminary.

Differences in ecology of these two species also remain unclear. So *Nardia pacifica* is characterized by Bakalin & Klimova (2016) as *"*unlike to the latter (*N. japonica*, N.K.), *N. pacifica* prefers well formed bryophyte synusia". However "fine soil along stream, stones near streams covered with thin soil" that is characteristic for *Nardia japonica* do not contradict occurring of "well formed bryophyte synusia". In many specimens preserved in KPABG which we earlier referred to *N. japonica,* the species occurs mixed with other bryophytes, similar to sequenced specimens of *N. pacifica* both from Alaska and the Caucasus where the latter species occurs in stiff mats of other liverworts on grained sandy soil. All this leaves open the question of the distribution of these species, particularly it is not clear to which species the numerous records of *N. japonica* from the European and Asian parts of Russia belong. For a more or less correct solution to this issue, it requires the study of living material and/or at least selective DNA sequencing of specimens.

The genus *Nardia* occupies an isolated position and was recently allocated to a separate subfamily Nardioideae Váňa in the family Gymnomitriaceae (Váňa *et al*., 2014). The genus was placed in this family as a result of modern molecular phylogenetic studies of the largest families of previously accepted suborder Jungermanniinae – Lophoziaceae, Scapaniaceae, Gymnomitriaceae and Jungermanniaceae (Vilnet *et al.*, 2010, 2011). These studies (l.c.) included only three species of *Nardia*. Later, Shaw *et al.* (2015) tested nine species presented by twelve specimens and supported the results obtained earlier (l.c.) but stressed that the interspecific relationships within the genus remain unclear. The inclusion in our study of three *N. pacifica* specimens did not change the topology obtained earlier. Particularly, four specimens identified as *N. geoscyphus* turned out to locate in different clades. A comparative morphological study of the sequenced samples is necessary to decide which of them belong to the *Nardia geoscyphus* and to which species belong the samples from the second clade named as well *Nardia geoscyphus*. At this stage we can only support the conclusion of Shaw *et al*. (2015) that "expanded population level studies are needed to resolve species level relationships and evolutionary groups within the genus".

Acknowledgements

We thank A.N. Savchenko for processing and preparing photos for printing. A. Hagborg is gratefully acknowledged for linguistic corrections. The study was carried out within institutional research project of the Avrorin Polar-Alpine Botanical Garden-Institute, RAS NN 1021071612832-8-1.6.11

LITERATURE CITED

BAKALIN, V.A. & K.G. KLIMOVA. 2016. A note on *Nardia japonica* Steph. (Gymnomitriaceae). – *Botanica Pacifica* **5**(2): *43–50*.

GOLOBOFF, P.A. & S. CATALANO. 2016. T.N.T. version 1.5, including a full implementation of phylogenetic morphometrics. – *Cladistics* **32**: *221–238*. https://doi.org/10.1111/cla.12160

HALL, T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. – *Nucleic Acids Symposium Series* **41**: *95–98*.

HONG, W.S. & J. Váňa. 2000. The distribution of *Nardia* in western North America. – *Lindbergia* **25**: 9*–14.*

RAMBAUT, A. A.J. DRUMMOND, D. XIE, G. BAELE & M.A. SUCHARD. 2018. Posterior summarisation in Bayesian phylogenetics using Tracer 1.7. – *Systematic Biology* **67**: *901–904*. https://doi.org/10.1093/sysbio/syy032

RONQUIST, F., M. TESLENKO, P. VAN DER MARK, D.L. AYRES, A.DARLING, S. HÖHNA, B. LARGET, L. LIU, M.A. SUCHARD & J.P. HÜLSENBECK. 2012. MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. – *Systematic Biology* **61**: *539–542*. https://doi.org/10.1093/sysbio/sys029

[SCHLJAKOV, R.N.] ШЛЯКОВ Р.Н. 1981. Печеночные мхи Севера СССР. – [The Hepatics of the North of the USSR] *Л., Наука* [*Leningrad, Nauka*] **4**: *1–220.*

SHAW, B., B. CRANDALL-STOTLER, J. VÁŇA, R.E. STOTLER, M. VON KONRAT, J.J. ENGEL, E.C. DAVIS, D.G. LONG, P. SOVA & A.J. SHAW. 2015. Phylogenetic relationships and morphological evolution in a major clade of leafy liverworts (phylum Marchantiophyta, order Jungermanniales): suborder Jungermanniineae. – *Systematic Botany* **40**: *27–45*. https://doi.org/10.1600/036364415X686314

SHAW, J., E.B. LICKEY, J. BECK, S.B. FARMER, W. LIU, J. MILLER, K.C. SIRIPUN, C. WINDER, E.E. SCHILLING & R.L. SMALL. 2005. The tortoise and the hare II: relative utility of 21 noncoding chloroplast DNA sequences for phylogenetic analysis. – *American Journal of Botany* **92**: *142–166.* https://doi.org/10.3732/ajb.92.1.142

TABERLET, P., L. GIELLY, G. PAUTOU & J. BOUVET. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. – *Plant Molecular Biology* **17**: *1105–1109*. https://doi.org/10.1007/BF00037152

TAMURA, K., G. STECHER & S. KUMAR. 2021. MEGA11: Molecular Evolutionary Genetics Analysis version 11.– *Molecular Biology and Evolution* **38**: *3022–3027*. https://doi.org/10.1093/molbev/msab120

VÁŇA, J, L. SÖDERSTRÖM, A. HAGBORG, & M.J. VON KONRAT. 2014. Notes on Early Land Plants Today. 60. Circumscription of Gymnomitriaceae (Marchantiophyta). – *Phytotaxa* **183** (4): *287–289*

VILNET, A.A., N.A. KONSTANTINOVA, & A.V. TROITSKY. 2010 [2011]) Molecular insight on phylogeny and systematics of the Lophoziaceae, Scapaniaceae, Gymnomitriaceae and Jungermanniaceae. – *Arctoa* **19**: *31–50*.

VILNET, A.A., N.A. KONSTANTINOVA, & A.V. TROITSKY. 2011. Taxonomical rearrangements of Solenostomataceae (Marchantiophyta) with description of a new family Endogemmataceae based on *trn*L-F cpDNA analysis. – *Folia Cryptogamica Estonica* **48**: *125–133*.

Received 10 May 2022 *Accepted 27 May 2022*