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Phylogenetic Relationships among Members of the Subfamily Sedoideae (Crassulaceae) Inferred from the ITS Region Sequences of Nuclear rDNA

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Abstract—Nucleotide sequences of the nuclear rDNA ITS regions were determined in 20 species of the subfamily Sedoideae (Crassulaceae). The phylogenetic relationships of these species with other members of the subfamily, occurring mainly in Southeast Asia, were analyzed. It was shown that the genus *Orostachys* was not monophyletic; its type subsection was significantly included into the clade of the genus *Hylotelephium*. Synapomorphic substitutions and indels, specific for the subsection *Orostachys*, were detected in ITS1. Sister relationships were established between clades *Aizopsis* and *Phedimus*, based on which they can be recognized as isolated genera.

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INTRODUCTION

The family Crassulaceae DC. consists of species of predominantly succulent appearance, adapted to xerophytic conditions and characterized by specific crassulacean acid metabolism (CAM). Members of this family are nearly cosmopolitan, inhabiting largely semiarid and mountainous regions. The greatest variety of Crassulaceae is observed in Mexico, South Africa, Micronesia, and East Asia. Since its description, the family Crassulaceae was regarded as a natural taxon, which, after separation of the genus *Penthorum* L., was universally recognized as monophyletic [1, 2]. The family includes approximately 1300 species, classified into 33 genera. Because of high morphological diversity in the family Crassulaceae and marked geographical confinement of most of its genera, resulting in the presence of several evolutionary branches within the family, the infrafamilial classification has long been and still is under debate. The most widely recognized classification of this family is the Berger system [3], based on the characters of the number and positions of flower parts, the degree of sympetaly, leaf positions, and geographical distribution of the genera. According to this system, the family Crassulaceae includes six subfamilies. Note that many authors regarded this system as artificial, but all attempts to revise it proved unsuccessful [2, 4].

The key taxonomic problems of the family Crassulaceae concern generic and subgeneric classification of the subfamily Sedoideae Berger and its typical genus *Sedum* L. (s.l.) [5, 6]. Most of crassulacean species of Eurasia were at some point included in this genus, but later were isolated as distinct genera *Orostachys* (DC.)

Fisch. ex A. Berger, *Hylotelephium* H. Ohba, and *Aizopsis* Grulich, which occur mainly in East Asia and are probably of the East Asian origin.

The new systematics of the family, based on the results of early molecular phylogenetic studies, which confirmed the artificial nature of most Crassulaceae subfamilies and demonstrated convergent evolution of their morphological characters, suggested that part of the Asian members of the subfamily Sedoideae sensu Berger (genera *Hylotelephium*, *Orostachys*, *Phedimus* Rafin., *Pseudosedum* (Boiss.) Berger, *Rhodiola* L., *Sedum*, *Sinocrassula* Berger, and *Umbilicus* D.C.), forming clade *Telephium* in the phylogenetic tree, were isolated in the subtribe Telephiinae [2]. All species of the subtribe have pentamer flowers with free stamens and obdiplostemonous androecium (stamens are positioned in two circles, the external ones being accumbent to the petals, while the internal ones alternate with them). Note that, though this new system was recognized by some authors, the clade *Telephium* is not significant [5, 7, 8].

Recently, Mauryzumi and Ohba [6] attempted to establish the phylogenetic position of Asian Sedoideae on the basis of nuclear and chloroplast DNA sequences [6]. The authors confirmed independence of the crassulacean genera, separated from *Sedum* s.l.: *Rhodiola*, *Hylotelephium*, *Phedimus*, and *Umbilicus*, but failed to establish monophyly of the clade *Telephium*, comprising species of presumably East Asian origin. It was also noted that the natural character of this clade is problematic to substantiate from the point of view of morphology, because there is no unique (synapomorphic) char-

acters, distinguishing this clade from the remaining Sedoideae sensu Hart (Sedoideae). Moreover, it was shown that the major genera of this clade are not monophyletic. For instance, *Hylotelephium* together with some members of *Orostachys*, as well as *Rhodiola* and *Pseudosedum*, formed robust clades in the phylogenetic tree. Thus, the results of molecular phylogenetic studies raise doubts as to the natural character of these taxa and suggest revision of the generic boundaries in East Asian Crassulaceae.

Presumably non-monophyletic genera *Hylotelephium*, *Orostachys*, and *Rhodiola* form the basis of the Crassulaceae flora of the Russian Far East. In the present study, they are represented by respectively nine, eight, and eight species [9, 10]. Some of these taxa (*Rhodiola rosea*) are very widely spread, others, by contrast, are endemic for the Russian Far East (*Hylotelephium ussuriense*, *Orostachys paradoxa*).

Although the similarity of the species lists of these genera on the adjacent territories of North China, Korean Peninsula, and Japan is great, comparison of the species descriptions shows the absence of the universal approach to understanding many of the taxa [11]. As was aptly pointed by Kozhevnikov [12], "poor knowledge of Sedoideae in natural populations and their analysis largely based on sporadic collections led to the situation, when many of the species were described repeatedly, while the actual diversity of Sedoideae remains to be vague." Although this statement referred to Himalayan and Chinese species, it can be justly applied to the family as a whole.

In this work, we present the results of comparing the nucleotide sequences of the internal transcribed spacer (ITS region) of nuclear ribosomal DNA of members of the family Crassulaceae in order to establish their phylogenetic relationships.

MATERIALS AND METHODS

We used samples of 20 plant species, collected in various localities of Primorskii krai and grown in the collection of Botanical Garden Institute, Russian Academy of Sciences (table).

DNA isolation, amplification, and sequencing. Total cell DNA was extracted and purified using QIAGEN DNeasy Plant Mini Kit (QIAGEN, Hilden, Germany), according to the instructions of the manufacturer. The ribosomal operon region, encoding 3' end of 18S rDNA, internal transcribed spacer 1 (ITS1), 5.8S rDNA, ITS2, and 5' end of 28S rDNA were amplified in polymerase chain reaction (PCR), using primers N-nc 18S10 and C26A [13]. The PCR products were used for cyclic sequencing with the BigDye v. 3.1 kit (Applied Biosystems) and primers N18L18, N5.8S, ITS2, and ITS4 [13]. Sequencing of the both strands of the PCR products was performed in the ABI PRIZM 310 sequencer (Applied Biosystems). The sequences were assembled, using the Staden software package [14].

Sequence alignment and construction of phylogenetic trees. To analyze phylogenetic relationships among the members of the subfamily Sedoideae, we constructed a data matrix that included the newly derived and retrieved from the GenBank database sequences (table). Three species, of *Kalanchoe*, the genus close to Sedoideae [5–8], were taken as an out-group. The sequences were manually aligned by means of the SeaView program [15], following conserved elements of the primary and secondary structure of the ITS region [16].

Phylogenetic trees were constructed using the maximum likelihood (ML), neighbor-joining (NJ), and maximum parsimony (MP) methods, implemented in the phylogenetic program PAUP 4.0b10 [17], as well as the Bayesian inference (BI), implemented in the program MrBayes v3.0b3 [18]. Evolutionary models for the ML and NJ analyses were selected using the Modeltest 3.04 program [19]. Distances for the NJ analysis were computed via ML optimization. A heuristic search for optimal topology was used for the ML and MP analyses. In BI analysis, one million of Markov chain generations were created, taking probes every 100 generations, which yielded 10 000 probes. The first 500 probes (before $-\ln L$ values reached plateau) were excluded from analysis as "burn-in." Robustness (statistical support) of the NJ and MP phylogenetic trees was assessed by the bootstrap [20], using 1000 bootstrap replications and posterior probabilities (PP) in BI. The bootstrap percentage (BP) less than 50% and PP less than 0.90 were not considered and are not shown in the figure. In the bootstrap analysis of the MP trees, a heuristic search for the optimal topology was conducted with a random addition of taxa.

RESULTS

The length of the spacer region (ITS1, 5.8S, and ITS2) in the species examined varied from 597 base pairs (bp) in *Rhodiola yunnanensis* to 621 bp in *Hylotelephium tsugaruense* with a mean of 609.4 ± 4.2 bp and a typical for angiosperms content of G + C bases of $57.5 \pm 0.04\%$. In most genera, the length of ITS1 exceeded that of ITS2 (mean values 226 ± 2.3 and 219.4 ± 4.8 bp, respectively). Only the members of *Sinocrassula* and *Kalanchoe* Adans. showed the reverse trend in the spacer lengths. The GTR model of nucleotide sequence evolution, supplemented with the gamma-parameter (Γ) and the proportion of invariant positions (I) was shown to most adequately describe our dataset. The characteristic feature of these data was a high frequency of the following two types of nucleotide substitutions: C \longleftrightarrow T (4.6918) and A \longleftrightarrow G (2.4155). Of 604 bp used in the analysis, almost half (295 bp) were informative; 251 bp, invariant; and 58, MP uninformative.

The results of the ML analysis of 69 taxa and 604 bp are shown in Fig. 1. In the phylogenetic tree, the subfamily members were grouped in two clusters, which

Sedoideae species and GenBank numbers of the rDNA region ITS sequences

Species	Number	Species	Number
<i>Aizopsis</i> (<i>Phedimus</i>) <i>aizoon</i> (L.) Grulich.	AB089767	<i>K. delagoensis</i> Eckl. et Zeyh.	AJ231306
<i>A. (Phedimus) aizoon</i> var. <i>floribundus</i> (Nakai) H. Ohba	AB088614	<i>K. streptantha</i> Berger	AJ231322
<i>A. hybridum</i> (L.) Grulich	AM039908	<i>Meterostachys sikokiana</i> (Makino) Nakai	AB088579
<i>A. (Phedimus) kamtschatica</i> (Fisch.) Grulich	AB088612	<i>Orostachys aggregata</i> (Makino) Hara	AB088574
<i>A. litoralis</i> (Kom.) S. Gontch.	AM039909	<i>O. fimbriata</i> (Turcz.) Berger	AB088578
<i>A. middendorffiana</i> ssp. <i>sichotensis</i> (Vorosch.) S. Gontch.	AM039913	<i>O. furusei</i> Ohwi	AM039921
<i>A. x pilosa</i> S. Gontch. et Koldaeva	AM039910	<i>O. gorovoi</i> Dudkin et S. Gontch.	AM039922
<i>A. selskiana</i> (Regel et Maack) Grulic (Khasan L.)	AM039911	<i>O. iwarenge</i>	AB088573
<i>A. selskiana</i> (Khanka L.)	AM039912	<i>O. maximowiczii</i> Byalt	AM039923
<i>A. sikokiana</i> (Maxim.) Grulich	AB088613	<i>O. japonica</i> (Maxim.) Berger	AB088576
<i>Hylotelephium anacampseros</i> (L.) H. Ohba	AB088563	<i>O. malacophylla</i> (Pall.) Fisch.	AB088572
<i>H. angustum</i> (Maxim.) H. Ohba	AB088560	<i>O. malacophylla</i>	AM039924
<i>H. cauticola</i> (Praeger) H. Ohba	AB088566	<i>O. paradoxum</i> (Khokhr. et Vorosch.) Czerep.	AM039925
<i>H. cyaneum</i> (J. Rudolf) H. Ohba	AM039914	<i>O. spinosa</i> (L.) C.A. Mey	AB088577
<i>H. erythrostictum</i> (Miq.) H. Ohba	AB088556	<i>Phedimus spurius</i> (M. Bieb.) H. t'Hart	AB088616
<i>H. ewersii</i> (Ledeb.) H. Ohba	AB088570	<i>P. stellatus</i> Rafin.	AM039926
<i>H. pallescens</i> (Freyn) H. Ohba	AB088558	<i>Pseudosedum longidentatum</i> Boriss.	AB088609
<i>H. pallescens</i>	AM039915	<i>Pseudosedum</i> sp.	AB088610
<i>H. pluricaule</i> (Kudo) H. Ohba	AM039916	<i>Rhodiola amabilis</i> (H. Ohba) H. Ohba	AB088587
<i>H. populifolium</i> (Pall.) H. Ohba	AB088571	<i>R. angusta</i> Nakai	AM039927
<i>H. pseudospectabile</i> (Praeger) S.H. Fu	AM039917	<i>R. crenulata</i> (Hook f. et Thoms.) H. Ohba	AY359897
<i>H. triphyllum</i> (Haw.) Holub	AM039918	<i>R. fastigiata</i> (Hook f. et Thoms.) S.H. Fu	AB088594
<i>H. sieboldii</i> (Sweet. et Hook.) H. Ohba	AB088567	<i>R. ishidae</i> (Miyabe et Kudo) Hara	AB088600
<i>H. sieboldii</i> var. <i>ettyuense</i> (Tomida) H. Ohba	AB088568	<i>R. kirilowii</i> Regel ex Maxim.	AB088601
<i>H. sordidum</i> (Maxim.) H. Ohba	AB088562	<i>R. macrocarpa</i> (Praeger) S.H. Fu	AB088590
<i>H. spectabile</i> (Boreau) H. Ohba	AB088575	<i>R. rosea</i> L.	AB088599
<i>H. tatarinowii</i> (Maxim.) H. Ohba	AB088557	<i>R. yunnanensis</i> (Franch.) S.H. Fu	AB088602
<i>H. telephium</i> subsp. <i>maximum</i> (L.) H. Ohba	AB088561	<i>Sinocrassula indica</i> Berger	AB088580
<i>H. tsugaruense</i> (Hara) H. Ohba	AB088565	<i>S. paoshingensis</i> (S.H.Fu) H. Ohba et al.	AB088581
<i>H. ussuriense</i> (Kom.) H. Ohba	AM039919	<i>S. paoshingensis</i> var. <i>spinulosa</i> H. Ohba et al.	AB088583
<i>H. verticillatum</i> (L.) H. Ohba	AB088564	<i>S. yunnanensis</i> Berger	AB088582
<i>H. viride</i> (Makino) H. Ohba	AB088569	<i>Umbilicus botryoides</i> Hochst. ex A Rich.	AB088586
<i>H. viviparum</i> (Maxim.) H. Ohba	AB088559	<i>U. horizontalis</i> (Gruss.) DC.	AB088585
<i>H. viviparum</i>	AM039920	<i>U. rupestris</i> (Salisb.) Dandy	AB088584
<i>Kalanchoe campanulata</i> Baill.	AJ231309		

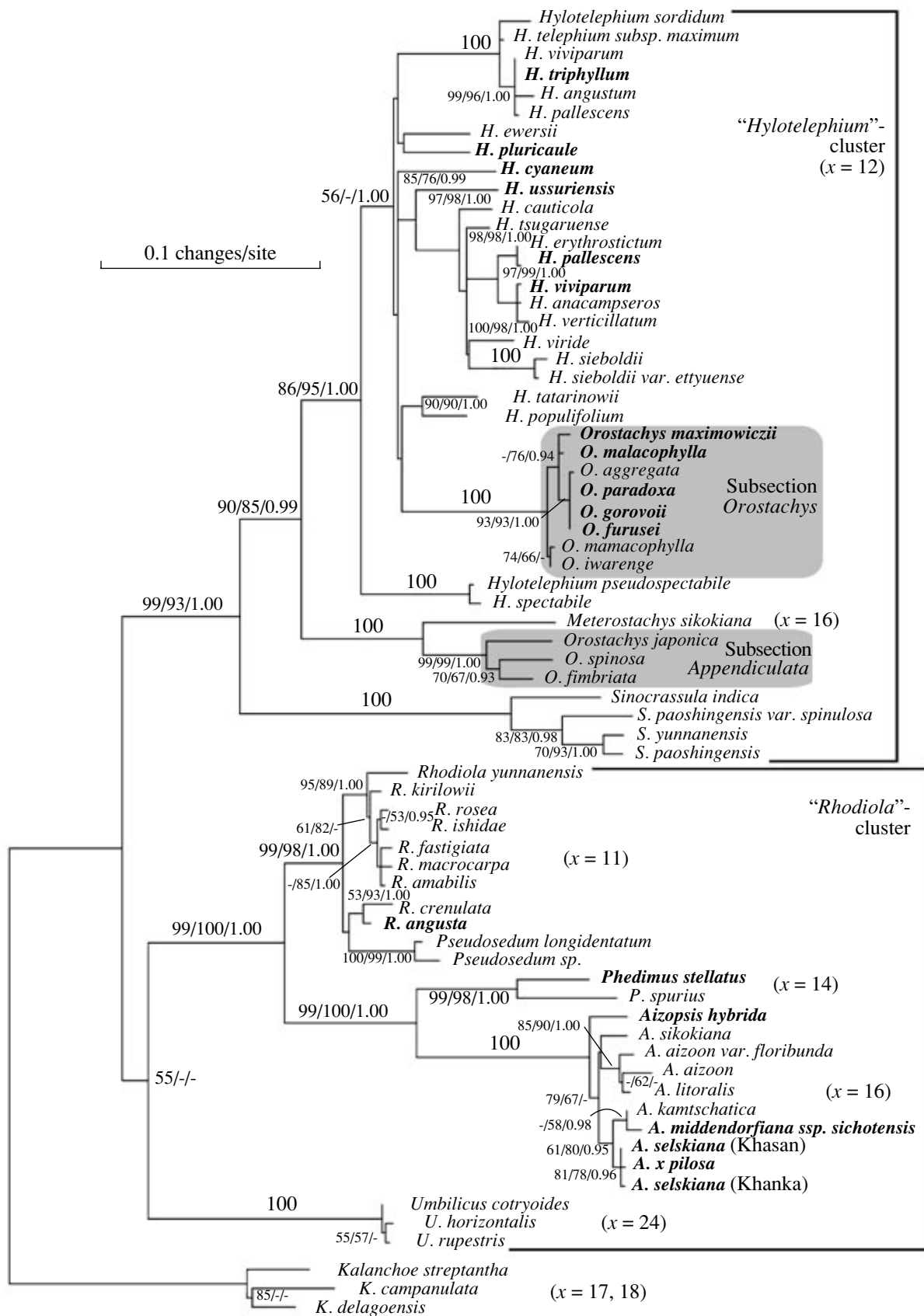
Note: Species with sequences determined in the present study are set out in bold. The genus names, given in GenBank database but not recognized by the authors of the present study, are given in brackets.

we conventionally termed *Hylotelephium* and *Rhodiola* groups. In the first, well supported (>93% BP, 1.00 PP) clade, the genus *Sinocrassula* formed a basal branch, which was followed by the robust (100% BP) sister pair *Meterostachys*–*Orostachys* subsection *Appendiculatae* (Boriss.) H. Ohba, while the *Hylotelephium* clade, reliably

(>85% BP, 1.00 PP) including members of *Orostachys* from subsection *Orostachys*, was at the crown.

The assignment of the basal genus *Umbilicus* to the *Rhodiola* group was supported by a low bootstrap value only in the NJ analysis. This genus was followed by two robust (99–100% BP, 1.00 PP) clades, each of which

Fig. 1. Phylogenetic tree of the 69 subfamily Sedoideae members, based on comparing rDNA ITS sequences by the maximum likelihood method (GTR + I + Γ model). Robustness of the trees was estimated for NJ/MP/BI analyses by means of bootstrap (NJ and MP) and posteriori probabilities (BI). Species with sequences determined in the present study are set out in bold. Two independent *Orostachys* clades are shown in gray. The basic chromosome numbers of clades (genera) are given in brackets.



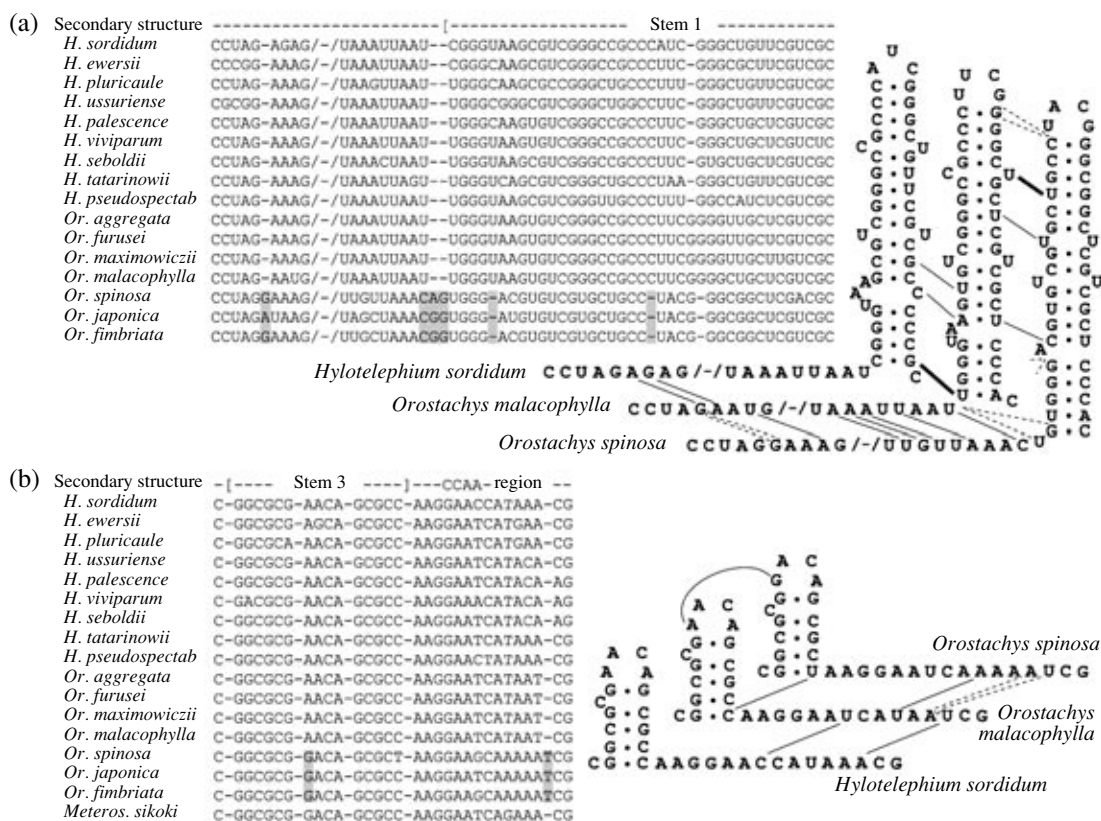


Fig. 2. Examples of synapomorphic indels and substitutions (shown in gray) in ITS1, distinguishing subsections of the genus *Orostachys*. Elements of the secondary structure of the spacer (a, stem 1; b, stem 3) are shown in accordance to model, predicted for ITS1 of *Rhodiola rosea* [16]. Coadaptive substitutions in stem 1 are marked by a bold line; indels are shown by a dashed line.

consisted of members of two genera: the *Phedimus*–*Aizopsis* pair and the clade of species of the genus *Rhodiola*, which included two *Pseudosedum* taxa. This topology characterizes as monophyletic only four out of eight genera studied (*Phedimus*, *Aizopsis*, *Umbilicus*, and *Sinocrassula*); their clades are supported by high bootstrap values (98–100%) and posteriori probabilities (1.00). The divergence among these clades is pronounced, and they all had long individual branches. Although differences between sequences in the clades are not substantial, they allowed determining the order of relationships among most of the species with high significance. *Sinocrassula*, *Phedimus*, and *Hylotelephium* showed the highest intrageneric divergence, while the genera *Rhodiola* and *Aizopsis*, by contrast, exhibited small differences among the species (Fig. 1).

Comparison of the branch lengths in two clades of the genus *Orostachys* showed that the among-species differences in the subsection *Appendiculatae* were pronounced and their sequences clearly differ from one another (25–35 substitutions), whereas in the subsection *Orostachys*, the differences did not exceed 2–4 bp. These clades differed not only in the number of nucleotide substitutions and the G + C content (61.1 ± 0.05 and $56.0 \pm 0.02\%$, respectively), but also in the pres-

ence of specific indels (Fig. 2), which were not taken into account in phylogenetic analysis. The highest number of indels was found in more variable ITS1. Interestingly, clades of the subsections virtually did not differ in the length of this pacer and the whole spacer region, i.e., deletions in some ITS parts were compensated by insertions in its other parts.

In our study, some species (*Hylotelephium viviparum*, *H. palescence*, *A. selskianum*, and *Orostachys malacophylla*) are represented by sequences from two specimens. Only in the case of *A. selskianum*, no differences between these sequences were found. The specimens of *O. malacophylla* differ by four substitutions, while the differences between the specimens of *Hylotelephium viviparum* and *H. palescence* are even greater, exceeding 50 substitutions and indels. Not unexpectedly, these sequences are located in different parts of the clade of the genus (Fig. 1). Given the generally high divergence among the *Hylotelephium* species, this genus also has several significant species groups, the members of which display high similarity of the ITS sequences (\leq ten substitutions). These are pair *H. erythrodictum*/*H. palescence*, clade *H. viviparum*/*H. anacampseros*/*H. verticillatum* and the robust *H. sordidum* clade that includes six sequences (Fig. 1).

DISCUSSION

Comparison of the nucleotide sequences of the internal transcribed rDNA spacer in 69 members of ten genera from the subfamily Sedoideae, occurring mostly in Asia, allowed us to establish their phylogenetic relationships. The results of this analysis generally conformed to the previously reported evidence, inferred from smaller data sets and other markers [6]. We have focused on analysis of the relationships among the members of genera *Orostachys* and *Hylotelephium*, whose monophyly had been in doubt [6], as well as genera *Phedimus* and *Aizopsis*, which some authors regard as synonyms [1, 21].

Orostachys is thought to be the most morphologically distinct member of the subfamily [22]. This genus is characterized by semi-rosette habit and terminal, narrowly pyramidal to cylindrical in outline inflorescences. The genus has two sections, *Orostachys* and *Schoenlandia* [22], the latter of which, recognized by some authors as a subgenus [23] or a distinct genus *Kungia* Fu [24], is absent in the Russian Far East. The species of the type section are classified into two subsections, *Orostachys* and *Appendiculatae*, which differ by leaf shape (flat or fleshy) and the presence of absence of leaf appendages (spines, cartilaginous protrusions, etc.) Molecular phylogenetic data supported the closeness of the species from each subsections, but established the absence of relatedness between the subsections. Moreover, the type subsection *Orostachys* was significantly ($> 85\%$ BP, 1.00 PP; Fig. 1) placed among the members of the *Hylotelephium* genus. These relationships are problematic to substantiate from the morphological viewpoint, but congruence of phylogenies, inferred from nuclear (the present study) and the chloroplast [6] genomes, confirms their significance. The characters shared by *Hylotelephium* and *Orostachys* are leaf morphology (flat leaves without appendages) and gynecium, but the latter taxon is distinguished by semi-rosette biomorphs, dicyclic monocarpic shoots, and pyramidal inflorescences.

Orostachys species with fleshy leaves with cuspidate tips (subsection *Appendiculatae*) are also grouped into a robust clade. This morphological synapomorphy may have higher taxonomical significance than thought earlier. This conclusion is supported by the relationships between *Orostachys*, subsection *Appendiculatae* and the monotypic genus *Meterostachys* (Fig. 1), which also has fleshy leaves with spines.

Apparently, the genera *Hylotelephium* and *Orostachys* are close (Fig. 1) and may have a common ancestor, which is evidenced also by the equal basic chromosome number in the two genera ($x = 12$ [26]). However, the structure of their clade contradicts the traditional composition of the genera, implying necessity of taxonomic rearrangements. Thus, a recognition of the clade *Hylotelephium*–*Orostachys*, subsection *Orostachys*, based on the results of molecular phylogenetic analyses (the present study; [6]) implies that all members of the clade

should be included into the priority genus *Orostachys* [25]. This means that the currently existing genus diagnosis must be altered in order to cover the morphological diversity of the novel combined genus. To date this is hardly possible, because no characters are known that would be common for all of the species of such genus and distinguish them from species outside the genus.

According to the resultant topology, *Orostachys*, subsection *Appendiculatae* should be recognized as a distinct genus. The issue of assigning *Meterostachys sikokiana* to this taxon remains open. The sister relationships between the taxa are not at variance with either the independence of the latter or the possibility of their combination. The resolution of this issue depends on the weight assigned to the characters shared by the clade members and distinguishing them from the other members of Sedoideae. In our view, *Meterostachys* rather clearly differs from the members of subsection *Appendiculatae* by the obligatory rosette biomorph, auxiliary panicle inflorescences, and the basic chromosome number ($x = 16$ and 12 , respectively [26]). At the molecular level, the difference lies in the fact that *Meterostachys* lacks an insertion in the ITS1 CA region, characteristic of representatives of *Orostachys*, subsection *Appendiculatae* (Fig. 2b).

The topology of our phylogenetic tree poses a question on the isolation of another two taxa, the genera or subgenera *Phedimus* and *Aizopsis*. Until recently, they were considered sections or subgenera within the genus *Sedum* [3, 22], but later were recognized as genera [11, 27, 28]. Even the early molecular phylogenetic studies showed similarity of these taxa (represented by one species each); consequently, they were both included into the genus *Phedimus* [2]. In the our tree, the genera *Phedimus* and *Aizopsis* are sister clades, each of which includes the type species of the corresponding genus (*P. stellatus* and *A. aizoon*, respectively; Fig. 1). This topology permits either recognition of these clades as genera or the possibility of their integration into one genus. We believe that the differences between the clade members in coloration (pink or while in *Phedimus* and yellow in *Aizopsis*) and morphology of flowers, in the distribution (predominantly European in *Phedimus* and Asian in *Aizopsis*), and in the basic chromosome number ($x = 14$ and 16 , respectively [26, 29]) are significant and differentiate them as distinct genera.

The presented phylogeny includes most species of the genus *Hylotelephium*, ranking second in the number of species in the subfamily Sedoideae, which permits evaluation of the current intrageneric system. This taxon includes two sections and two subsections, differing by life forms and the orientation of shoots. Describing these taxa, Ohba [22] emphasized the morphological similarity of their species, supposing that it reflects their common origin. However, molecular phylogenetic data indicate that all these subgenera and sections are artificial and suggest that characters characterizing them arose independently. Robust clades are distin-

guished within the traditional genus *Hylotelephium*, but to date the morphological characters uniting their members are hard to find. Discordance between the traditional within-genus classification and the molecular phylogenetic evidence was also noted for the genus *Rhodiola* [6].

Analysis of the distribution of life forms in the clades implies their repeated independent appearance in various Sedoideae and does not enable to determine, which of them was ancestral in the subfamily. Earlier, it was proposed that “originally herbaceous” plants may be original in the family Crassulaceae, giving rise to “soft woody” and typical “woody” (having developed secondary xylem and specialized tectorial tissues) forms [30]. Among the analyzed species, the anatomical structure close to the hypothetical ancestor is characteristic only to *Phedimus stellatus*, positioned as the crown branch of the *Phodiola* cluster. This position in the tree can indicate either conservation of the ancestral character state and a secondary simplification resulting from “turning herbaceous” (term from [31]).

It seems very likely that long-shooted life forms, characteristic of the *Hylotelephium* species, originated from semi-rosette ones. The tree topology shows that the presence of long shoots (rather than rosette and semi-rosette ones, as thought earlier) is an autapomorphy for Far Eastern Sedoideae [7]. Life forms with such shoots are typical for the basal genus *Sinocrassula*, clades *Orostachys*, and *Meterostachys sikokiana*.

The preservation of another plesiomorphic [7] character, kyphocarpic carpels, in the *Phedimus/Aizopsis* clade still has no conclusive explanation. The apomorphy of ortocarpic carpels in Crassulaceae is regarded as evident [22], but on the presented phylogenetic tree the species with plesiomorphic state of this character occupy a crown position, which casts doubt on the latter conclusion.

Thus, our study confirmed the presence of significant controversy between the traditional views on evolution and the phylogenetic relationships of East Asian Sedoideae. Further research is required to reliably establish the taxonomic structure of the subfamily and natural boundaries of the genera, as well as positions of these taxa in the family.

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