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DNA extraction from old herbarium material of *Veronica* subgen. *Pseudolysimachium* (*Plantaginaceae*)

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Abstract. Herbarium specimens have become a major source of information in molecular biodiversity research, framing the term "herbarium genomics". However, obtaining good DNA from old herbarium specimens is still a challenge. Currently, DNA extraction methods from old herbarium material often yield highly degraded and fragmented DNA. A number of studies have discussed such methods, especially how to avoid further DNA fragmentation. This study aims to compare different DNA extraction methods applied to old herbarium material from *Veronica* subg. *Pseudolysimachium*. One such method is a CTAB-based DNA extraction followed by a clean-up with paramagnetic beads that is used in the Jodrell Laboratory, Royal Botanic Gardens Kew, UK. This method was compared to a modified NucleoSpin Plant II protocol, based on silica columns, as used at the Technical University Munich-Freising, which was already successfully used for extracting DNA from a Linnean type specimen. Further tests were conducted on the influence of incubation time on the CTAB DNA extraction protocol with a subsample of specimens. Our preliminary results suggest that CTAB DNA extraction might have some advantages in specific cases but also that silica column-based methods have fewer problems with contamination by polysaccharides and polyphenolic compounds. Regarding the incubation time, we did not observe a clear pattern, but we developed several ideas on how to proceed with tests to find an optimal DNA extraction protocol to deal with highly fragmented DNA. Taking practical considerations into account, the column-based method proves to be preferable, especially when trying to reduce the amount of leaf tissue used, but further modifications of both methods should be explored.

Keywords: *Veronica* subg. *Pseudolysimachium*, herbarium specimens, DNA extraction methods, molecular biodiversity research

Supplementary Material. Electronic Supplement (Table E1, p. e3) is available in the online version of this article at: https://ukrbotj.co.ua/archive/75/6/564

Introduction

Research on natural biodiversity is often obscured by being forced "to work in suboptimal conditions that include inadequate preservation methods, limited sampling regimes, and suboptimal tissue type and quantity" (Blair et al., 2015: 1079–1080). Nevertheless, it is possible to overcome the problem of inaccessibility of specimens by using herbarium material, which is a valuable source of DNA (Staats et al., 2011), to clarify a multitude of various questions. However, the bigger problem is that herbarium DNA is usually highly degraded and modified (Staats et al., 2011) and that the small amounts of DNA can be a limiting factor in downstream applications like high-throughput sequencing (= HTS; Lovmar, Syvänen, 2006; Lasken, 2009). Therefore, several methods have been developed for DNA extraction of herbarium specimens (Záveská Drábková, 2014), which sometimes deal with taxon-specific problems and sometimes have taxon-specific success rates.

In our project to investigate the status of several "microspecies" of *Veronica* subg. *Pseudolysimachium* (W.D.J.Koch) Buchenau from the eastern part of

Ukraine, for which a plethora of names have been described (Klokov, 1976; Tzvelev, 1981; Ostapko, 1984, 1994, 2014; Ostapko et al., 2010), herbarium material is a vital resource since it proves difficult to collect new material, especially for Donetsk Region (*Oblast* in Ukrainian), Luhansk Region, and the Crimean Peninsula due to the current political and military situation. Therefore, this project will focus on herbarium material and, furthermore, demonstrate the feasibility to retrieve DNA from type material to clarify the relationships of the described species and infraspecific entities.

Therefore, we compared DNA extraction methods from two labs, the Jodrell Laboratory at the Royal Botanic Gardens (Kew, UK), and the laboratory of Prof. Dr. Hanno Schaefer, Technical University Munich-Freising, (Germany). Both labs have extensive experience in DNA extraction from herbarium specimens (e.g., Dodsworth, 2015; Schaefer et al., 2009; Dwivedi et al., 2018). We were particularly interested in exploring those methods used in regular molecular biology laboratories without specialized ancient DNA facilities, which requires a lot of time, money, and institutional support (Knapp et al., 2012).

DNA extraction at Kew was formerly conducted using a "macroprep protocol" as described in Albach and Chase (2001). This protocol is based on the strong detergent cetyltrimethyl ammonium bromide (CTAB) (Kistler, 2012), which has been the "gold standard" for plant DNA extractions since the mid-1980s (Doyle, Doyle, 1987; Rogers, Bendich, 1985). This is followed by a DNA cleaning step using chloroform and isoamyl alcohol for removing proteins (Albach, Chase, 2001) and an isopycnic ultracentrifugation in cesium chloride, meaning that the molecules will be separated according to their density. This method has typically been used to separate the plastid/mitochondrial DNA from nuclear DNA (Carr, Griffith, 1987) to avoid analyzing numts (= nuclear mitochondrial DNA segment, DNA which was transferred from the mitochondrial to the nuclear genome) since they present a different evolutionary fate (Arthofer et al., 2010). While this yields ultra-pure DNA suitable for long-term storage especially recommendable for DNA from type material, Kew has replaced the expensive and time-consuming technique for many projects in favor of a modified CTAB DNA extraction protocol similar to the original CTAB protocol proposed by Doyle and Doyle (1990). The protocol was modified by subsequent cleaning with Solid Phase Reversible Immobilization beads (= SPRI), which are paramagnetic beads, i.e. they are just magnetic in a magnetic field. These beads are polystyrene coated with a layer of magnetite and carboxyl molecules on top, which can reversibly bind DNA in the presence of polyethylene glycol (a crowding agent) and salt (DeAngelis et al., 1995).

This method was compared with a modified "NucleoSpin II Plant" (Macherey-Nagel Inc., Düren, Germany) mini DNA extraction protocol (Schaefer et al., 2009; Dwivedi et al., 2018). This commercial kit was already successfully used in a study to extract DNA from a Linnean type specimen (Chomicki, Renner, 2015). In this respect, it should be noted that a number of different commercial plant DNA extraction kits based on silica-membrane methods are available. These kits do differ in specifics and may, therefore, differ in their suitability for DNA extraction from herbarium specimens and/or different taxa, which we noticed in preliminary tests (Dirk C. Albach, unpublished data). Finally, we explored the effect of extending the incubation time since different taxa work better with different incubation times during lysis (Edgardo M. Ortiz, unpublished data; Drábková et al., 2002).

Numerous other modifications have been reported to improve DNA extraction from herbarium specimens, such as PTB-buffer extraction (Kistler, 2012) and an extended precipitation (Staats et al., 2011). Another alternative is whole genome amplification such as the "Restriction and Circularization-Aided Rolling Circle Amplification" (= RCA-RCA) method described by Wang et al. (2004) that shows promising results when working with highly degraded template DNA, according to Blair et al. (2015). For a similar method multiple displacement amplification (MDA), feasibility has been already shown to produce enough DNA as a starting point for "restriction site associated" sequencing methods (Blair et al., 2015). The RCA-RCA method is considered to be superior to the already tested MDA since it does not produce non-specific amplification artefacts (Blair et al., 2015, also in the absence of input genomic DNA), as it was reported for MDA, which is crucial when working with low DNA input (Lage et al., 2003). The MDA method also faces problems with short fragments (Steven Dodsworth, unpublished data), which is congruent with findings that MDA is not able to replicate fragments below 1 kbp (Li et al., 2006; Maciejewska et al., 2013). However, testing of these additional methods is beyond the scope of the current project. Our preliminary results (see Höpke, Albach, 2018) were briefly reported at the International Conference 'Herbaria and Phytodiversity Conservation' (3–5 October 2018, Lviv, Ukraine). Here we provide a full report.

Material and Methods

Sampling material

The two DNA extraction methods were tested on samples from our focal taxonomic group *Veronica* subg. *Pseudolysimachium*. Samples were taken from the herbarium in Oldenburg and from leaf samples that had already been collected for DNA extractions (from herbarium sheets and silica dried leaves). The samples were chosen to fit two mini centrifuges, i.e. 48 samples, and to include a homogeneous sampling of all available decades of collection. From *Veronica* subg. *Pseudolysimachium,* sampling included two specimens available from the 1950s, two from the 1960s, nine from the 1970s, one from the 1980s, five from the 1990s, ten from the 2000s, and ten from the last decade. Since the availability of old material from this subgenus was restricted, we supplemented this with *Veronica* specimens from other subgenera as follows: two specimens from the 1950s, 11 from the 1970s, four from the 1980s, and three from the 1990s. From these samples, c. 20 mg of leaf tissue was taken for CTAB DNA extraction and 10 mg for column-based DNA-extraction with subsequent standardization of results.

We took additional samples for the incubation time analysis. For this, twice c. 10 mg leaf samples from ten herbarium sheets from different decades (each) was taken: one from the 1950s, one from the 1960s, two from the 1970s, one from the 1980s, one from the 1990s, two from the 2000s, and two from the last decade. With these, two different incubation times were tested during lysis. Information on all specimens used in the analysis can be found in the Electronic Supplement (Table E1.).

CTAB and bead clean-up

The preparation and DNA extraction were conducted in a sodium hypochlorite-cleaned fume hood (designated for DNA extraction only) with sodium hypochlorite-cleaned equipment and pipettes used with filter tips only. For the CTAB mini protocol, one liter of 2x CTAB buffer was prepared as follows: 12.11 g TRIS/TRIZMA (100 mM final concentration), was dissolved in a small amount of distilled water using an agitator and magnetic stirrer. To this 7.5 g EDTA (20 mM) was added, filled up with distilled water to 0.5 L, and adjusted to a pH of 8.0. Afterwards, 82 g NaCl (1.4 M), 20 g CTAB (2% w/v), and 20 g PVP (2% w/v) were added but dissolved one after another and finally filled up with distilled water to 1 L. Firstly, for the DNA extraction protocol, isopropanol and the blocks of the grinder were put into a freezer at 20 °C and a water bath was preheated to 65 °C. The lysis buffer was prepared by mixing 747 µL 2x CTAB buffer per sample with 3 µL of 2-mercaptoethanol (equaling 0.4% of the isolation buffer) and 3 µL of 10 mg/mL RNAse A. Each sample (20 mg) was then ground with two steel balls (c. 4 mm in diameter) within the precooled grinder blocks (to prevent overheating and enzymatic activity) at 25,000 rpm for 2 min using a Retsch MM400 (Retsch Inc., Haan, Germany). Immediately afterwards, 750 µL freshly prepared lysis buffer was added, vortexed for 3 sec and incubated for 30 min in a water bath at 65 °C. Every 5 min the samples were mixed by hand. Afterwards, 750 µL SEVAG (chloroform : isoamyl alcohol 24 : 1) was added, vortexed for 3 sec and the sample tubes were attached with a tape onto a shaker platform, here an Orbital Shaker Model SO3 (Cole-Parmer Inc., Stone, United Kingdom), running at 250 rpm. After 30 min, the samples were centrifuged at 13,000 rpm for 15 min, 550 µL of the supernatant above the protein pellet was transferred into a new 2.0 mL microcentrifugation-tube, 367 µL of −20 °C precooled isopropanol (2/3 of the supernatant volume) was added, the samples were vortexed for 3 sec and shortly centrifuged so that all droplets moved to the bottom of the tube and kept overnight in the freezer at −20 °C.

At the beginning of the next day Qiagen AE elution buffer (Qiagen Inc., Venlo, Netherlands) was preheated to 65 °C. Samples in isopropanol were centrifuged at 13,000 rpm for 15 min, the aqueous phase was decanted, the DNA pellet was washed by adding 750 µL of freshly prepared 70% ethanol, centrifuged at 13,000 rpm for 10 min, decanting the ethanol and washing it in the same way a second time. All ethanol was removed, the pellet was dried by placing the samples with opened lids in a GeneVac miVac Duo Concentration, operating with a Duo Pump (Thermo Fisher Scientific Inc., Waltham, MA, USA) at 46 °C for 5 min (drying overnight under the fume hood is also possible). Then, 100 µL of the preheated elution buffer was added to each sample, the pellet was thoroughly resuspended and shortly centrifuged, the samples were incubated at 65 °C in the water bath for 30 min, vortexed for 3 sec, and again shortly centrifuged.

The bead clean-up was conducted on a normal molecular lab workbench by adding two times the eluted DNA volume (200 µL) of the undiluted AMPure XP bead solution (Beckman Coulter, Brea, CA, USA) to the eluted DNA. This DNA-bead solution was mixed, spun down shortly and incubated at room temperature (RT) for 5 min. Afterwards the tubes were placed in a

12-tube magnetic separation rack for 1.5 mL tubes (New England BioLabs Inc., MA, USA). After 5 min or longer until the solution becomes clear and a pellet was formed, the supernatant was removed and discarded, and the pellet was washed twice by adding 300 µL of freshly prepared 80% ethanol, which was left for 30 sec before being removed. Subsequently all the ethanol was removed, the pellet dried for 5 min and thoroughly resuspended in 60 µL AE elution buffer, spun down shortly and incubated for 5 min at RT on a normal rack, and then for 5 min at RT on the magnetic rack. Finally, 50 µL of the aqueous phase containing the clean DNA was transferred to a new tube.

Quality control A

For the CTAB-bead DNA extraction, a QuantiFluor dsDNA System (Promega Inc., Fitchburg, WI, USA) was used to measure the dsDNA concentration. Here, 20x TE buffer was diluted to 1x before use. The standard was prepared with 2 µL standard solution, 98 µL 1x TE and 100 µL dye; the blank was prepared with 100 µL 1xTE and 100 µL dye, and the samples were prepared with 1 µL DNA solution, 99 µL 1xTE and 100 µL dye. A Quantus Fluorometer (Promega Inc., Fitchburg, WI, USA) was first calibrated using the standard and the blank before measuring the concentration of the samples. To measure the A260/A280 and A260/A230 absorbance ratios, a Nanodrop 2000 (Thermo Fisher Scientific, Waltham, MA, USA) was used by measuring 1 µL Qiagen AE elution buffer (Qiagen Inc., Venlo, Netherlands) for calibration and afterwards 1 µL DNA solution of each sample.

Extended incubation times

The effect of an extended incubation time was tested by incubating the samples in PHMT thermoshakers (Grant Instruments Inc., Cambridge, United Kingdom) at 400 rpm and 65 °C during lysis with one sample set for 2 h and the other one for 4 h to compare with our standard 30 minutes. Since these tests were conducted in a different lab, there were a number of differences apart from incubation times. First, HighPrep PCR clean-up beads (MagBio Inc., Gaithersburg, MD, USA) were used instead of AMPure XP beads. Furthermore, the non-heated PHMT thermoshaker was used with samples taped on top of it and running at 550 rpm to yield a similar shaking result when mixing samples with the SEVAG solution (compared to the Orbital Shaker SO3 at 250 rpm). Moreover, the samples were eluted using an elution buffer from a different company (Macherey-Nagel, Düren, Germany).

Column-based DNA extraction

Column-based DNA extraction was conducted in a 70% ethanol-cleaned fume hood with 70% ethanol-cleaned equipment and pipettes used with filter tips only. Samples (10 mg) were grinded at RT together with three steel balls (c. 3 mm in diameter) and half a 3 mm spoon of fine silica (< 0.5 mm) stepwise (to prevent overheating) with 1500 rpm for 20 sec using a Retsch MM400 (Retsch Inc., Haan, Germany). Overall, all samples needed at least 60 sec of grinding to yield a fine powder. Samples were stored at -20 °C until further use DNA was extracted using the NucleoSpin Plant II mini kit following the manufacturer's protocol (Macherey-Nagel, Düren, Germany) with SDS as a lysis buffer (using buffers PL2 and PL3) except for some small modifications: For cell lysis no RNAse was used and incubation was conducted at a slightly lower temperature (62 °C) with an increased incubation time (40 min) in a PHMT thermoshaker (Grant Instruments Inc., Cambridge, United Kingdom) at 500 rpm. For the clarification of the lysate the centrifugation time was increased to 5 min and no filter column was used (since the membrane often got blocked by the cell fragments). Instead the clear supernatant was transferred to a 1.5 mL tube and mixed with 350 µL binding buffer before loading on the column. Furthermore, centrifugation was increased to 2 minutes. DNA was eluted in two steps with 25 µL elution buffer. Finally, all remaining ethanol was allowed to evaporate thoroughly (c. 45 min).

Quality control B

Concentration of column-based dsDNA extracts were analyzed on a Qubit dsDNA HS assay kit with a dye/buffer premix (Thermo Fisher Scientific, Waltham, MA, USA). Standards 1 and 2 were prepared with 190 µL dye/buffer premix and 10 µL standard solution, each; the samples were prepared with 198 µL dye/buffer premix and 2 µL DNA solution, each. Both standards were used to calibrate the Qubit 4 fluorometer (Thermo Fisher Scientific, Waltham, MA, USA) before measuring each sample. For measuring the A260/A280 and A260/A230 absorbance ratios an Epoch Microplate Spectrophotometer (BioTek Inc., Winooski, VT, USA) was used by firstly calibrating the system using 2 µL of PE buffer (Macherey-Nagel, Düren, Germany), and then 2 µL of DNA solution per sample was measured.

Statistical analysis

To calculate the total dsDNA yield, the dsDNA concentration was multiplied by the final elution

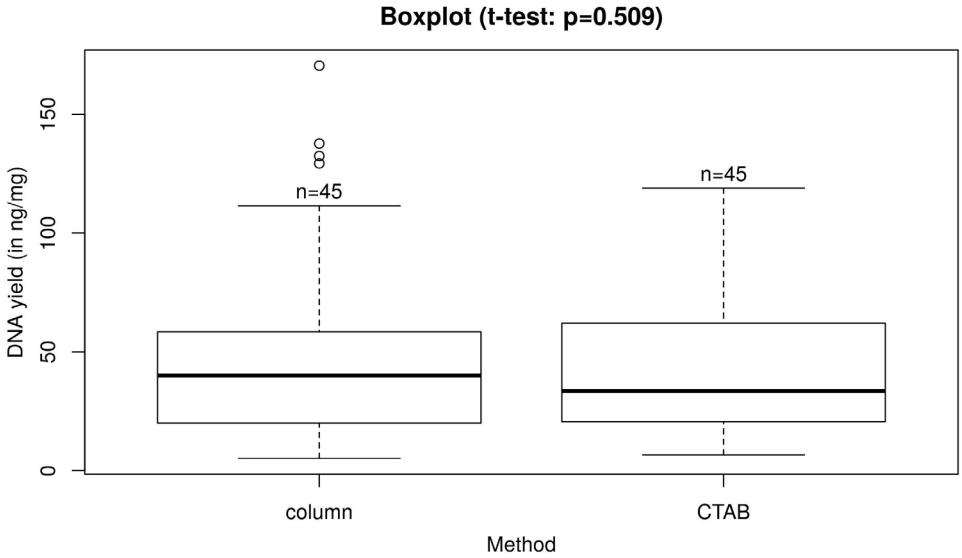

Fig. 1. Boxplot for DNA yield against extraction method (between column-based extraction and CTAB method)

volume. This value was then standardized by dividing the total dsDNA yield by the leaf dry mass used, which provided an estimate of the "DNA yield" (in ng of DNA per mg of used dried leaf tissue). For this parameter as well as the absorbance ratios A260/A280 and A260/A230 (after removing 'not available data') AN(C)OVAs were conducted to test the relationships between the year of collection, sample type, subgenus and extraction method before focusing on the latter. The Shapiro-Wilk test and Levene test were used to assess the AN(C)OVA assumptions for normality and homoscedasticity (Dormann, Kühn, 2009). Even though these assumptions were often not met, AN(C)OVAS were used since they are used for data-inspection. When the assumptions for normality and homoscedasticity were met, a Tukey-HSD posthoc test was done but when the assumptions were not met, additional Kruskal-Wallis rank-sum tests and the Dunn test that uses a Bonferroni p-value adjustment method as a posthoc test (Dunn, 1964) were conducted. For pairwise comparisons t-tests were used when AN(C)OVA assumptions were met, and if the assumptions were not met, a Wilcoxon rank sum test was conducted instead.

Results

DNA yield

DNA yield was not influenced by taxonomy, as indicated by a non-significant Dunn test ($\alpha > 0.05$) and inspecting the corresponding boxplot (result not shown).

Comparing the DNA yield of both methods excluding two extreme outliers each, for which apparently some DNA was lost along the way, a paired t-test did not reveal a significant difference ($p = 0.509$). The column based-method had only a higher median DNA yield value with 40.1 ng/mg and some additional high "outliers" (above the whiskers that are defined with 1.5 times the interquartile range), whereas the CTAB-method had a median value of 33.5 ng/mg (Fig. 1).

Since we were interested to know if the year of collection might have an influence on DNA yield (not found in the ANCOVAs), a scatterplot was used to visualize the relationship between the DNA yield of both methods and the year of collection. Although the assumptions for the two independent linear regressions were fulfilled, the adjusted R2 values for both are below 0.08 and the scatter plot also does not reveal any clear pattern (Fig. 2). Including values for some specimens extracted only with one method, a tendency for DNA yield being lower was observed in older material but the unbalanced sampling and the low adjusted R2 value (0.07) have to be kept in mind (Fig. 2).

DNA quality

The ANCOVA for A260/A280 did not meet the statistical assumptions (even after reducing the model to significant variables only) but implies that the year of collection ($p = 0.003$) and DNA extraction method ($p < 0.001$) have an important influence. Here,

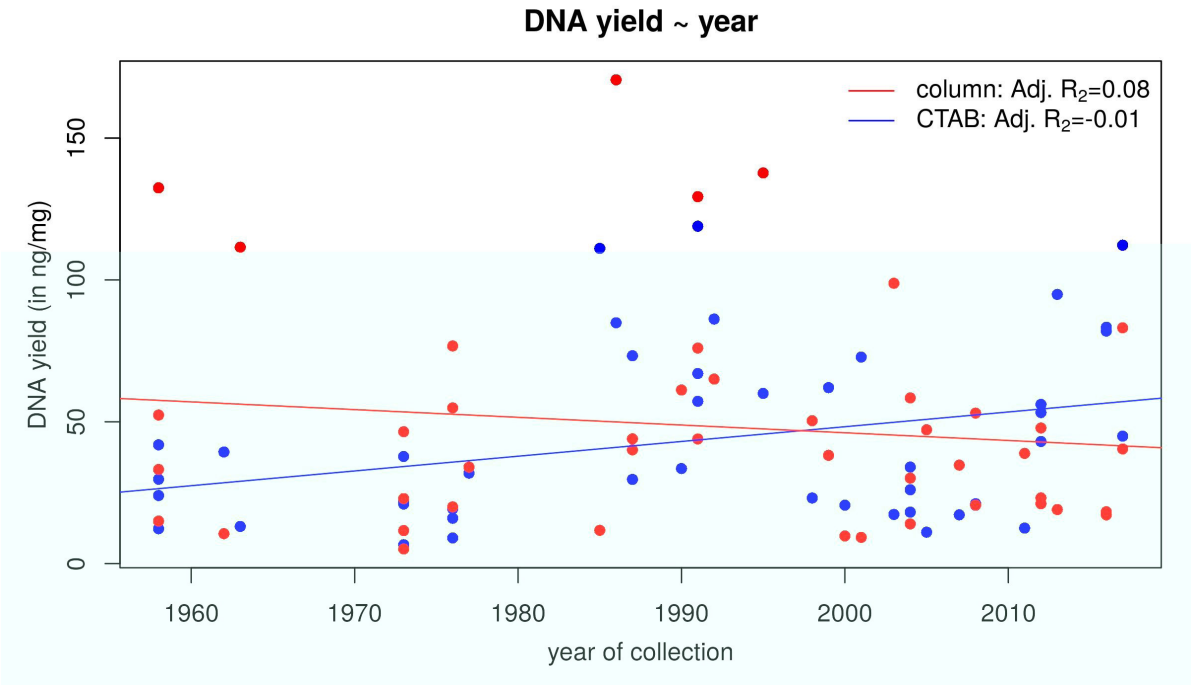

Fig. 2. Scatterplot for DNA yield against extraction method and year of collection (between CTAB and column-based extraction)

although the ANCOVA has a low R2 value, the plot shows that the A260/A280 ratio increases with the year of collection for both methods and that the column-based method is c. 0.1 above the CTAB-based method (Fig. 3A). Statistical assumptions were likewise not met for the ANCOVA of A260/A230, but it suggests that the extraction method ($p < 0.001$) is the only important variable. Furthermore, it shows, although weakly, that A260/A230 values for the two different sample types are different ($p = 0.06$). However, testing this explicitly using a Wilcoxon rank sum test (since the assumptions of a t-test were not fulfilled) no significant difference ($p = 0.1759$) was observed (Fig. 4).

For comparing the absorbance ratios, the previously removed observations were not removed here since they had no special placement in their scatter plots. Comparing both methods for A260/A280, a paired t-test showed they were significantly different. Here, column-based extraction showed a higher median ratio (2.04) than the CTAB method with 1.94. Also, the range of values for CTAB (1.54 to 2.24) was much wider than for column-based extractions (1.77 to 2.13) (Fig. 3B).

For A260/A230 values, a similar picture can be observed; although the difference is more extreme (e.g., the median of column-based extraction is 2.21 and for CTAB just 1.29). A paired t-test also showed this difference was significant ($p < 0.001$) (Fig. 5).

Incubation time

Investigating the influence of the prolonged incubation time for the CTAB-based DNA extraction protocol, it was shown that the DNA yield, as well as A260/A280 and A260/A230 decrease with increased incubation time. Interestingly, the decrease is not gradual. Instead, the middle incubation time (of 2 h) exhibits the minimum values for both DNA yield and A260/A280 ratios (Fig. 6).

Discussion

CTAB with bead clean-up vs. column-based DNA extraction

Statistical analysis did not reveal a significant difference in DNA yield between the two DNA extraction methods (Fig. 1), which were considered the most important criterion in this study. Other parameters seem to be more important such as initial sample drying process (Staats et al., 2011; Záveská Drábková, 2014) as well as the length of storage and fungi treatments during that time. Although initially counter-intuitive, we did not find a correlation of DNA yield with age. However, such a relationship was also not found in other studies (Choi et al., 2015; Shepherd, 2017), suggesting that DNA is stable if the plants are well stored and dried appropriately to start with.

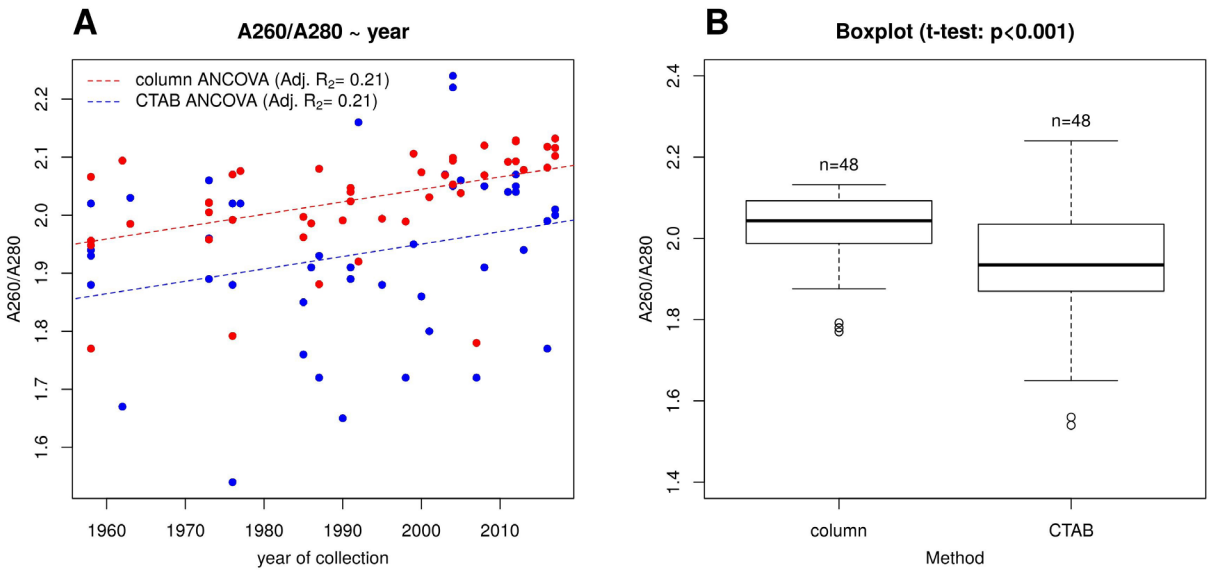

Fig. 3. Comparison of results for A260/A280 against extraction method (between CTAB and column-based method)
A: ANCOVA for extraction method and year of collection as covariate; B: Boxplot for A260/A280 against extraction method

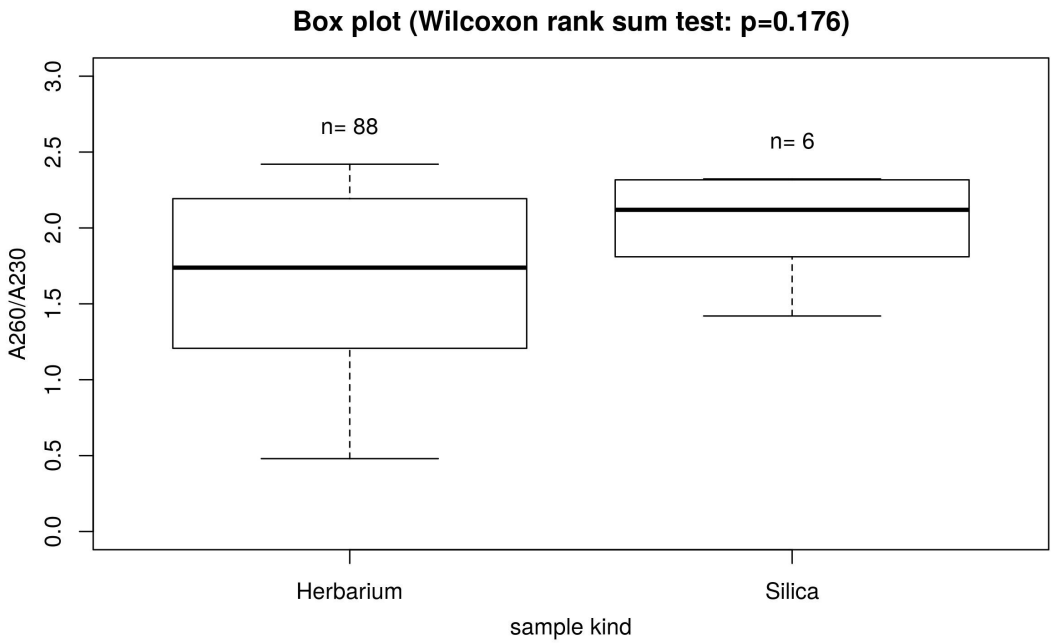

Fig. 4. Boxplot for A260/A230 against sample kind (for both DNA extraction methods combined)

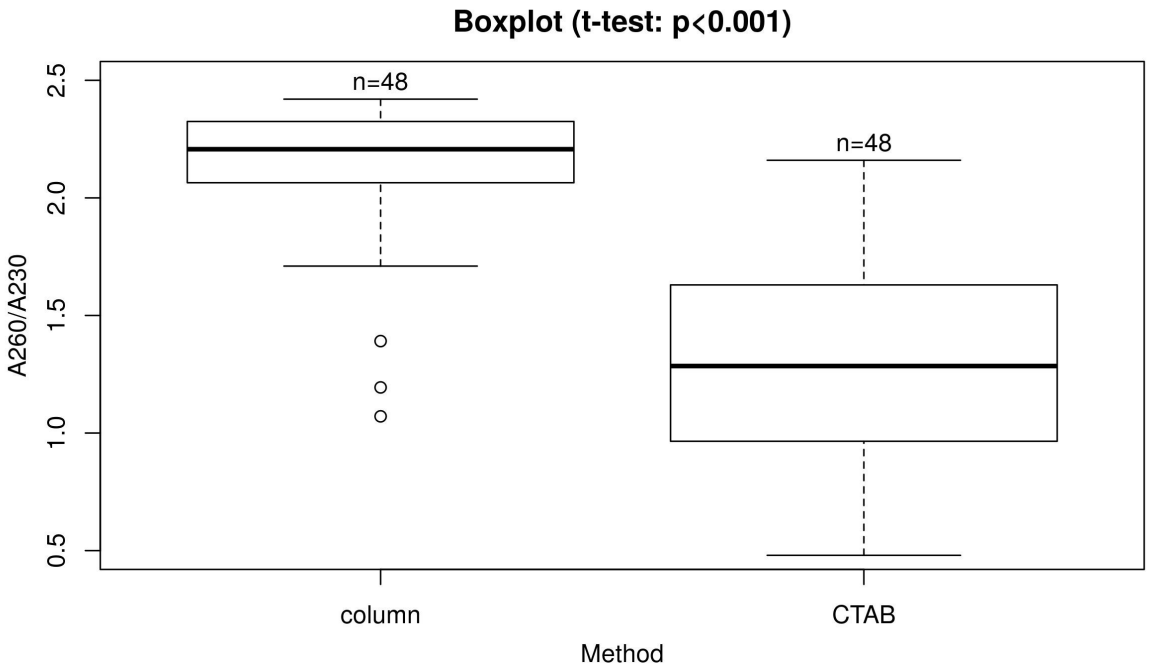

Fig. 5. Boxplot for A260/A230 against extraction method (between CTAB and column-based extraction)

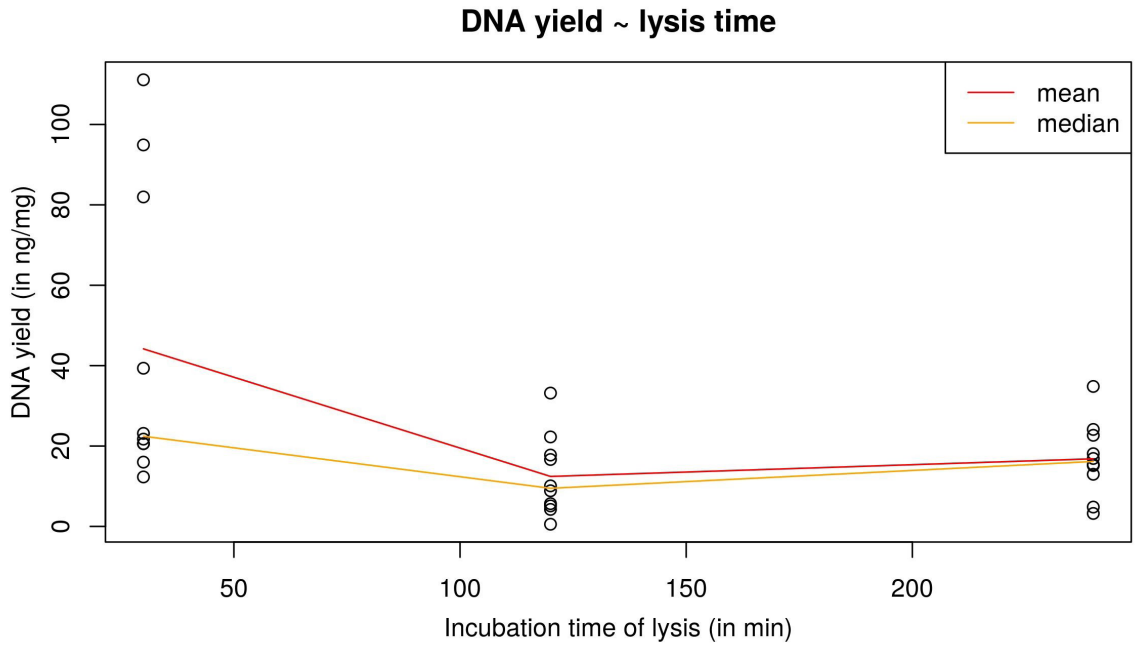

Fig. 6. DNA yield of the CTAB method against varying incubation times

Contrastingly, the DNA quality differed significantly between the two DNA extraction methods. As shown in the full sample set and subsample set, the DNA solutions from the column-based DNA extraction method were much purer than those from the CTAB-based protocol despite the extra cleaning step with paramagnetic beads. In the CTAB-based protocol, the A260/A280 values were not markedly below 1.8, thus indicating no contamination by proteins, phenols or other contaminants that absorb strongly near 280 nm. However, the A260/A230 values were markedly below 2.0, which indicates a contamination with EDTA, carbohydrates and/or other phenols that absorb near 230 nm (Anonymous, 2013). This corresponds to previous observations that polysaccharides (e.g., cellulose) and polyphenolic compounds can often not be removed in CTAB protocols (Turaki et al., 2017; Kenyon et al., 2008). The higher purity of DNA extracted using column-based kits is supposed to be the result of more stringently washing since the DNA is captured by a glass fiber filter (OPS Diagnostics, 2018, https://opsdiagnostics.com/notes/protocols/spin_column_plant_protocol.html).

An effect not considered thus far is that small fragments (below 100 bp) may be lost in column-based methods altogether as demonstrated in preliminary results (not shown). Previous studies have shown different results whereby smaller fragments (below 70 bp) were found to be retained (Anonymous, 2008). Additionally, Dabney et al. (2013) were able to sequence fragments as short as 30 bp using a modified silica column-based protocol by Rohland and Hofreiter (2007). These short fragments are commonly included in studies of humans but may be less helpful in studies of species without a sequenced genome.

Furthermore, these short fragments are often excluded in size-selective DNA purification steps (Dabney et al., 2013). However, this purification step is absent in a recently developed single-stranded library preparation method (Meyer et al., 2012), thus making it possible to use 30 bp long fragments (and with further improvements, potentially also 20 bp long fragments) according to Dabney et al. (2013). An important consideration when dealing with old type material is how to reduce the amount of tissue used since 20 mg leaf material may be highly destructive for small herbarium samples.

Different incubation times

Even though prolonged incubation times might increase the DNA yield and work better for other taxa (Drábková et al., 2002), this study found to the contrary. It was observed that after 2 h and 4 h incubation time, some samples included pigments (after the CTAB protocol but before the bead clean-up), which indicates an increase in contaminants since this was not the case when testing with a 30 min lysis step.

Nevertheless, the fact that DNA yield, A260/A280 and A260/A230 were all lower after 2 h in comparison to 30 min and 4 h indicates problems in this experiment. The reason for this might be that during the bead clean-up (after the ethanol washing) the pellets were air-dried under the fume hood with slightly different durations (for 5 min after 2 h lysis and 10 min after 4 h lysis). Thus, it seems to be advantageous to wait slightly longer to assure that all ethanol evaporated. However, the different bead solution used may be sufficient to explain this pattern. Future comparisons should aim at reducing these variables, which may seem slight but could be important regarding the little amount of DNA present in the specimens.

Improving the comparison

DNA extraction is a destructive method and sufficient yield requires sufficient amounts of starting material. Therefore, methods have been developed to reduce the destruction such as suggestions to rub material from leaves rather than use whole leaves (Shepherd, 2017). If herbarium specimens are partly destructed, one will guarantee that the material is used most efficiently, although there is a trade-off. We noticed that the CTAB protocol becomes difficult to process with just c. 10 mg of leaf tissue since the DNA pellet became too small and too translucid to see if a pellet formed on the side of the tubes. Therefore, methods have been developed that more efficiently release DNA from the tissue (Kistler, 2012).

Apart from absolute DNA amounts recoverable from herbarium specimens, such DNA is often also highly degraded to short fragments. There are various steps in the DNA extraction that may degrade DNA even further. For example, paramagnetic bead-aided methods might increase the probability that DNA breaks due to mechanical forces and 2-phase DNA extractions might be an alternative (Mayland-Quellhorst, personal communication). Thus, there are obvious ways to improve DNA extraction and commercial companies have started to advertise such methods, such as "MagPure

Plant DNA LQ Kit" (Biotech Angen Inc., Guangzhou, China), which needs just one centrifugation step after lysis to transfer the DNA containing supernatant into a new tube in which it is cleaned with paramagnetic beads. Even different commercial DNA extraction kits may result in different DNA yield and quality results (Albach, Dodsworth, unpublished data).

Another aspect deserving further investigation is whether DNA impurity as measured by a low 260/230 ratio is a problem for library preparation and DNA sequencing.

Further improvements are especially needed for controlling the intra-sample variation; therefore, we would need to collect larger quantities of leaf tissue per sample, grind these larger quantities, mixing it thoroughly and making equal aliquots of the leaf powder that are stored at -20 °C before testing different DNA extraction methods.

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Хьопке Я.[1], Брюер Г.[2], Додсворт С.[2,3], Ортіс Е.М.[4], Альбах Д.К.[1] **Виділення ДНК зі старих гербарних зразків представників *Veronica* subgen. *Pseudolysimachium* (*Plantaginaceae*).** Укр. бот. журн., 2018, 75(6): 564–575.

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Гербарні зразки стали важливим джерелом інформації для молекулярних досліджень біорізноманіття, і навіть виник термін "гербарна геноміка". Проте, отримання хороших зразків ДНК зі старих гербарних зразків все ще є складним завданням. На даний час методи екстракції ДНК зі старого гербарного матеріалу часто дозволяють отримати лише деградовану або фрагментовану ДНК. Такі методи обговорювалися у багатьох дослідженнях, зокрема, щодо вирішення проблеми подальшої фрагментації ДНК. Метою нашого дослідження було порівняння різних методів екстракції ДНК зі старих гербарних зразків представників *Veronica* subg. *Pseudolysimachium*. Один з цих методів – екстракція ДНК на основі CTAB (цетилтриметиламоній бромід або цетил-триметил-бромід амонію) з наступним очищенням за допомогою парамагнітних гранул, що використовується у Лабораторії Джодрелла у Королівському ботанічному саду К'ю (Велика Британія). Цей метод порівнювався з модифікованою методикою NucleoSpin Plant II на основі силікагелевих колонок, що використовувалася у Технічному університеті Мюнхен-Фрайзінг (Німеччина) і була успішно застосована для отримання ДНК з типового зразка гербарію К. Ліннея. Проводилися подальші тести з вибіркою зразків щодо впливу часу інкубації на методику виділення ДНК за допомогою CTAB. Наші попередні результати свідчать, що CTAB-метод екстракції ДНК може мати певні переваги у конкретних випадках, але також вказують на те, що методи на основі силікагелевих колонок мають менше проблем із забрудненням полісахаридами та поліфенольними сполуками. Ми не виявили певної закономірності щодо часу інкубації, але розробили декілька ідей про те, як рухатися далі з експериментами для виявлення оптимальної методики екстракції ДНК для зразків, що містять фрагментовану ДНК. З практичного погляду, метод на основі колонок виглядає кращим, особливо тоді, коли є потреба зменшити кількість тканини листків. Проте, слід розробляти подальші вдосконалені модифікації обох методів.

Ключові слова: *Veronica* subg. *Pseudolysimachium*, гербарні зразки, методи екстракції ДНК, молекулярні дослідження

Хёпке Я.[1], Брюэр Г.[2], Додсворт С.,[2,3] Ортис Э.М.[4], Альбах Д.К.[1] **Выделение ДНК из старых гербарных образцов представителей *Veronica* subgen. *Pseudolysimachium* (*Plantaginaceae*).** Укр. бот. журн., 2018, 75(6): 564–575.

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Гербарные образцы стали важным источником информации для молекулярных исследований биоразнообразия; возник даже термин "гербарная геномика". Однако получение хороших образцов ДНК из старых гербарных образцов все еще является сложной задачей. В настоящее время методы экстракции ДНК из старого гербарного материала позволяют получить лишь деградированную или фрагментированную ДНК. Такие методы обсуждались во многих исследованиях, в частности, при решении проблемы дальнейшей фрагментации ДНК. Целью нашего исследования было сравнение различных методов экстракции ДНК из старых гербарных образцов представителей *Veronica* subg. *Pseudolysimachium*. Один из этих методов – экстракция ДНК на основе CTAB (цетилтриметиламмоний бромид или цетил-триметил-бромид аммония) с последующей очисткой с помощью парамагнитных гранул используется в Лаборатории Джодрелла в Королевском ботаническом саду Кью (Великобритания). Этот метод сравнивали с модифицированной методикой NucleoSpin Plant II на основе силикагелевых колонок, которая использовалась в Техническом университете Мюнхен-Фрайзинг (Германия) и была успешно применена для получения ДНК из типового образца гербария К. Линнея. Дальнейшие тесты проведены с выборкой образцов по влиянию времени инкубации на методику выделения ДНК с помощью CTAB. Наши предварительные результаты свидетельствуют о том, что CTAB-метод экстракции ДНК может обладать определенными преимуществами в конкретных случаях, но также указывают на то, что методы на основе силикагелевых колонок имеют меньше проблем с загрязнением полисахаридами и полифенольными соединениями. Мы не обнаружили определенной закономерности относительно времени инкубации, но разработали несколько идей о том, как двигаться дальше с экспериментами по выявлению оптимальной методики экстракции ДНК для образцов, содержащих фрагментированную ДНК. С практической точки зрения, метод на основе колонок является лучшим, особенно, когда необходимо уменьшить количество ткани листьев. Однако, следует разрабатывать дальнейшие усовершенствованные модификации обоих методов.

Ключевые слова: *Veronica* subg. *Pseudolysimachium*, гербарные образцы, методы экстракции ДНК, молекулярные исследования

Table E1. Information on specimens used in the analysis

Extraction_method	Incubation_lysis_min	Full_sample_set	Reduced_sample_set	Genus	Species	Subgenus	External_no	Collector	Collection_id	Herbarium	Sample_kind	Date_of_collection	Country	Locality_habitat	Latitude	Longitude	Altitude_m	Ploidy	Dry_weigth_mg	DNA_conc_flourometer_ng_ul	a260a230a260a280DNA_conc_spectrometer_ng_ulDNA_conc2_flourometer_ng_ul_just_for_F_Incubation time 2h_B2_different
CTAB	30	yes	no	*Veronica*	*barrelieri* subsp. *andrasovszkyi*	*Pseudolysimachium*	BP757299	Barina et al.	21604	BP	SILICA	2012-06-24	Albania	Diber, Komsi	41.57501N	19.94322E			21,6	23	1,422,0566,723
CTAB	30	yes	no	*Veronica*	*barrelieri* subsp. *andrasovszkyi*	*Pseudolysimachium*	BP761126	Barina & Pifko	22646	BP	SILICA	2012-06-23	Albania	Puke, Mt. Terbosh	42.00849N	19.91709E			20,9	18	1,812,0783,518
CTAB	30	yes	no	*Veronica*	*barrelieri* subsp. *andrasovszkyi*	*Pseudolysimachium*	BP768015	Barina et al.	23897	BP	SILICA	2012-06-24	Albania	Shkoder, south of Vau i Dejes Reservoir	42.05792N	19.68168E			19,6	22	1,982,0461,722
CTAB	30	yes	no	*Veronica*	*dahurica*	*Pseudolysimachium*	ALTB	Ebel A.L., Verhozina A.V., Mihaylova S.I., Strelnikova T.O., Sheremetova S.A., Hrustaleva I.A., Murashko V.V.	48448	ALTB	HERBARIUM	2017-08-17	Russia	Zabaykalskiy krai. Nerchinsko-Zavodskiy distr., near village Nerchinskiy-Zavod, riverbank Urov. Meadow steppe at the foot of the slope	N051°52'31.08"	E120°00'06.20"			20,2	82	1,962255,982
CTAB	30	yes	no	*Veronica*	*dahurica*	*Pseudolysimachium*	ALTB	Ebel A.L., Verhozina A.V., Mihaylova S.I., Strelnikova T.O., Sheremetova S.A., Hrustaleva I.A., Murashko V.V.	48450	ALTB	HERBARIUM	2017-08-13	Russia	Zabaykalskiy kray. Karyimskiy district, near village Zhimbira. Steppe slopes	N051°31'02.74"	E114°01'34.74'			19,6	44	1,692161,744
CTAB	30	yes	no	*Veronica*	*dahurica*	*Pseudolysimachium*	ALTB	Ebel A.L., Verhozina A.V., Mihaylova S.I., Strelnikova T.O., Sheremetova S.A., Hrustaleva I.A., Murashko V.V.	48453	ALTB	HERBARIUM	2017-08-16	Russia	Zabaykalskiy kray. Aleksandrovo-Zavodskiy district, 13 km south- east from village Aleksandrovskiy Zavod. Nerchinskiy mountain range. Left Bank of the river Malaya Borzya, South-West the slope of the mountain Murashiha. Grass-steppe meadow	N051°48'14.30"	E118°02'42.27"			17,8	16	1,732,0181,916
CTAB	30	yes	no	*Veronica*	*incana*	*Pseudolysimachium*	ALTB	G.V. Matyashenko	34171	ALTB	HERBARIUM	1977-08-08	Russia	Irkutskaya region, Olkhonskiy district, Olkhon island, Cape Kobyl'ja Golova (Chorin-Irgi). The North-Western slope	N053°03'44.00"	E106°54'14.00"			15,2	9,7	1,682,02107,99,7
CTAB	30	yes	no	*Veronica*	*incana*	*Pseudolysimachium*	ALTB	G.V. Matyashenko	36885	ALTB	HERBARIUM	1976-08-01	Russia	Irkutskaya oblast, Olkhonskiy district, lake Baikal, Bay Tashkay, South-East of village Tashkay. The top of the mountain, stone fields, steppe	N053°02'37.00"	E106°28'27.00"			9,9	3,83	1,332,0238,63,83
CTAB	30	yes	no	*Veronica*	*incana*	*Pseudolysimachium*	ALTB	L.N. Tyulina	43461	ALTB	HERBARIUM	1958-08-13	Russia	Irkutskaya oblast, Olkhonskiy district, lake Baikal, the North-Western coast of Baikal, Cape Pokoyniki. Pebble shore shaft at the lake	N054°00'31.00"	E108°12'58.00"			17,7	8,5	1,652,02105,28,5
CTAB	30	yes	no	*Veronica*	*incana*	*Pseudolysimachium*	ALTB	L.N. Tyulina	48524	ALTB	HERBARIUM	1958-08-02	Russia	Irkutskaya oblast, Olkhonskiy district, lake Baikal, the North-Western coast of Baikal, Cape Onguren. Rocky steppe slope to the lake	N054°00'31.00"	E108°12'58.00"			19,1	16	1,481,9316516
CTAB	30	yes	no	*Veronica*	*incana*	*Pseudolysimachium*	ALTB	M.G. Azovsky	46249	ALTB	HERBARIUM	1987-07-07	Russia	Irkutskaya oblast, Olkhonskiy district, vicinities village Ozera. Meadow steppe	N052°42'00.14"	E106°21'57.90"			8	4,75	1,051,9324,64,75
CTAB	30	yes	no	*Veronica*	*incana*	*Pseudolysimachium*	ALTB	S.G. Kazanovsky	48525	ALTB	HERBARIUM	1999-08-05	Russia	Irkutskaya oblast, Olkhonskiy district, lake Baikal, Malomorskoe coast, 1 km West of cape Ulibra. Stony steppe					13,7	17	1,721,9596,617
CTAB	30	yes	no	*Veronica*	*arenosa*	*Pseudolysimachium*	ALTB	S.A. Dyachenko.	No.1	ALTB	HERBARIUM	2005-06-24	Mongolia	Khobdo aimak, Mongolian Altai, NE macroslope of Arshantyn-Nuruu range, vicin. oft he spring Ulyastajn-Sala; Stony slopes, meadow steppes, Caragana & Artemisia communities.	46°22′ N	91°14′ E	1900		20,3	4,5	0,692,06594,5
CTAB	30	yes	no	*Veronica*	*arenosa*	*Pseudolysimachium*	ALTB	S.A. Dyachenko & P.A. Kosachev.	No.4	ALTB	HERBARIUM	2004-06-24	Mongolia	Khobdo distr., Mongolian Altai, Shadzgaityn-Nuruu range, N macroslope, valley of the left tributary of Ulyastajn-gol; meadow and steppe slopes	46°21′ N	91°41′ E			20,1	7,3	0,522,2449,57,3
CTAB	30	yes	no	*Veronica*	*arenosa*	*Pseudolysimachium*	ALTB	S.A. Dyachenko & P.A. Kosachev.	No.5	ALTB	HERBARIUM	2004-07-01	Mongolia	Khobdo distr., Mongolian Altai, middle reaches of Uenchijn-gol; stony slopes, limestone rocks	46°22′ N	92°07′ E			19,2	10	0,642,2259,710
CTAB	30	yes	no	*Veronica*	*laeta*	*Pseudolysimachium*	ALTB	Shmakov A. I., Ebel A. L. Kashcheev, M., Dyachenko S., Soloviev A.	No.2	ALTB	HERBARIUM	1991-07-21	Kazakhstan	Taldy-Kurganskaya oblast, Dzungarian Alatau, Kapal'skiy district, the upper river Sarkand, steppe slope of Western exposure					20,6	49	1,091,89102,449
CTAB	30	yes	no	*Veronica*	*laeta*	*Pseudolysimachium*	ALTB	Shmakov A. I., Ebel A. L. Kashcheev, M., Dyachenko S., Soloviev A.	No.4	ALTB	HERBARIUM	1991-07-21	Kazakhstan	Taldy-Kurganskaya oblast, Dzungarian Alatau, Kapal'skiy district, the upper river Sarkand, steppe slope of Western exposure					19,4	26	11,8986,126
CTAB	30	yes	no	*Veronica*	*laeta*	*Pseudolysimachium*	ALTB	Shmakov A. I., Ebel A. L. Kashcheev, M., Dyachenko S., Soloviev A.	No.5	ALTB	HERBARIUM	1991-07-21	Kazakhstan	Taldy-Kurganskaya oblast, Dzungarian Alatau, Kapal'skiy district, the upper river Sarkand, steppe slope of Western exposure					20,1	23	1,121,91100,723
CTAB	30	yes	no	*Veronica*	*alatavica*	*Pseudolysimachium*	ALTB	Laz'kov G.A.	s.n.	ALTB	HERBARIUM	2004-08-10	Kirgizstan	Kungey Ala-too, river Chok-Aksu					19,1	13	1,792,05118,913
CTAB	30	yes	no	*Veronica*	*grisea*	*Pseudolysimachium*	ALTB	V. Byalt, V. Nikitin, I. Illarionova, V. Shaulo & al.	No. 415	ALTB	HERBARIUM	2003-07-01_02	Russia	Tuva, Dzun-Khemchiksky distr., road Kyzyl-Shagonar, rv. Shemi. Sands and pebbles on banks	51°16′ N	91°11′ E	766		17,3	6	1,492,07105,16
CTAB	30	yes	yes	*Veronica*	*altaica*	*Pseudolysimachium*	OLD00641	Kamelin, R.V.; Shmakov, A.I.; Dorofeev, V.I.; Smirnov, S.V.; Chubarov, I.; Antonjuk, E.; Kosachev, P. & Kuzev, M.G.	s.n.	OLD	HERBARIUM	2000-07-02	Russia	Altaiskii krai, Krasnoschekovskii district, vicinity village Kharlovo, right bank river Charysh	51°41′ N	82°49′ E			22,1	9,1	1,241,861169,1
CTAB	30	yes	yes	*Veronica*	*barrelieri* (likely: *steppacea*)	*Pseudolysimachium*	OLD02924	Stepura (?)	126539	OLD	HERBARIUM	1962-06-15	Ukraine	Donetsk Region, Novoazovskiy District, Khomutovsty Steppe Nature Reserve					21,6	17	0,831,67229,117
CTAB	30	yes	yes	*Veronica*	*chamaedrys*	*Chamaedrys*	OLD00661	Buldakova; Paschenko	s.n.	OLD	HERBARIUM	1985-07-03	Russia	Altaiskii krai, Kurjinskii district, vicinity village 8 Marta, lake Beloe					13,5	30	1,091,85146,930
CTAB	30	yes	no	*Veronica*	*chamaedrys*	*Chamaedrys*	OLD00662	Buldakova; Paschenko	s.n.	OLD	HERBARIUM	1985-07-04	Russia	Altaiskii krai, Kurjinskii district, mountain Sinjucha; mixed forest					6,2	1,99	0,481,7635,11,99
CTAB	30	yes	no	*Veronica*	*chamaedrys*	*Chamaedrys*	OLD00663	Tamilova	s.n.	OLD	HERBARIUM	1987-06-22	Russia	Altaiskii krai, vicinity of station Povaliha; marge					11,6	17	0,881,727617
CTAB	30	yes	no	*Veronica*	*chamaedrys* subsp. *chamaedrys*	*Chamaedrys*	OLD00524	Mikoláš, V.	7083	OLD	HERBARIUM	1992-08-06	Slovakia	Slovakia centralis, Západné Tatry Mts., Zuberec village, Javorina Mt. (S of Osobitá Mt.)	49°15'23"N	19°43'23"E	1530		17,4	30	1,372,16140,830
CTAB	30	yes	no	*Veronica*	*chamaedrys* var. *villosa* Mikoláš ined.	*Chamaedrys*	OLD01107	Mikoláš, V.	16018	OLD	HERBARIUM	1986-05-01	Slovakia	Slovakia orientalis, Košice, Botanical Garden of University of P.J.Šafárika, at terraces	48°44'07"N	21°14'10"E	280		15,9	27	0,921,91171,527
CTAB	30	yes	no	*Veronica*	*densiflora*	*Stenocarpon*	OLD00664	Kamelin, R.V.; Shmakov, A.I.; Dorofeev, V.I.; Goljakov, P.; Solovjev, A.; Kascheev, M. Luchkin, E. & Smirnov, S.	s.n.	OLD	HERBARIUM	1995-07-06	Russia	Republic Altai, Kosh-Agachskii district, Chikhacheva ridge, pass Bugusun; alpine meadows	50°11′ N	89°25′ E			12,5	15	1,411,8894,115
CTAB	30	yes	yes	*Veronica*	*filifolia*	*Pentasepalae*	OLD02902	Kotov, M. and Omeltchuk, T.	s.n. Dups.: 05/10	OLD	HERBARIUM	1958-05-28	Ukraine	West Transcaucasia, Novorosijsk, Range Markotch; on the limestone					18,6	4,58	1,181,9460,54,58
CTAB	30	yes	yes	*Veronica*	*incana*	*Pseudolysimachium*	OLD02906	Klokov, M.	126570 Dups.: 05/10	OLD	HERBARIUM	1973-07-30	Ukraine	Kyiv Reg., station Klavdiievo; Pine-wideleaf forests					19,8	8,6	1,521,96938,6
CTAB	30	yes	no	*Veronica*	*incana*	*Pseudolysimachium*	OLD02907	Klokov, M. and Dubovik, O.	s.n. Dups.: 03/07	OLD	HERBARIUM	1973-07-18	Ukraine	Kyiv Reg., Irpin'; Pine-wideleaf forestes the other side of the river Irpin					19,9	20	0,771,56186,420
CTAB	30	yes	no	*Veronica*	*incana*	*Pseudolysimachium*	OLD02909	Tkatchenko, V.	s.n. Dups.: 07/07	OLD	HERBARIUM	1963-07-04	Ukraine	Lugansk Reg., Stanichno-Luganske; Steppe slopes on the right shore of the Severskyi Donetz River					20,2	5,3	1,612,03117,15,3
CTAB	30	yes	no	*Veronica*	*kiusiana*	*Pseudolysimachium*	OLD00365	Albach, D.C.	1060	OLD	HERBARIUM	2008-06-02	Korea	Botanischer Garten Mainz; cultivated from seeds ex Botanical Garden Yeomiji, Korea					21,3	9	1,521,9177,59
CTAB	30	yes	no	*Veronica*	*kiusiana* var. *maxima*	*Pseudolysimachium*	OLD00213	Albach, D.C.	1040	OLD	HERBARIUM	2007-05-29	Germany	cultivated at Botanical Garden Mainz, ex BG Bonn, XX-0-Bonn-22013				2	18,3	6,3	0,931,7248,26,3
CTAB	30	yes	no	*Veronica*	*linariifolia*	*Pseudolysimachium*	OLD00370	Albach, D.C. & Zhao, L.	1214	OLD	HERBARIUM	2011-07-17	China	Shaanxi prov., Ningshan County; outside Jiankou; open vegetation next to fields	33.653N	108.646E	800		19	4,76	1,142,0424,54,76
CTAB	30	yes	yes	*Veronica*	*maeotica*	*Pseudolysimachium*	OLD01194	Albach, D.C., Thielen, N., Mosyakin, S., Mosyakin, A.	1486	OLD	HERBARIUM	2016-07-01	Ukraine	Oblast Kiew: Koncha Zaspa meadows; clearing in pine forest margin, mossy	50°19,796′ N	30°34,064′ E	102	2	12,2	20	1,921,9955,220
CTAB	30	yes	no	*Veronica*	*paczoskiana*	*Pseudolysimachium*	OLD02915	Klokov, M. and Zoz, I.	s.n. Dups.: 07/07	OLD	HERBARIUM	1976-07-28	Ukraine	Kcharkiv Reg., Zmiivskyi Distr., villae Skrypaji; Pine forest					22,6	4,1	0,641,541874,1
CTAB	30	yes	no	*Veronica*	*paczoskiana*	*Pseudolysimachium*	OLD02916	Klokov, M. and Dubovik, O.	s.n. Dups.: 03/38	OLD	HERBARIUM	1973-07-20	Ukraine	Station Maljutinka near Kyiv sity; Pine forest					19,3	2,57	1,221,8924,72,57
CTAB	30	yes	no	*Veronica*	*paczoskiana*	*Pseudolysimachium*	OLD02919	Klokov, M. and Dubovik, O.	s.n. Dups.: 28/38	OLD	HERBARIUM	1973-07-20	Ukraine	Station Maljutinka near Kyiv sity; Pine forest					22,5	17	2,162,06181,617
CTAB	30	yes	no	*Veronica*	*paczoskiana*	*Pseudolysimachium*	OLD02923	Klokov, M. and Dubovyk, O.	126441 Dups.: 38/38	OLD	HERBARIUM	1973-07-20	Ukraine	near Kyiv (Kiev), Malyutynka railway station; Pine forest					20,7	8,7	1,081,8986,98,7
CTAB	30	yes	yes	*Veronica*	*paczoskiana*	*Pseudolysimachium*	OLD02925	Klokov, M. and Zoz, I.	126428	OLD	HERBARIUM	1976-06-25	Ukraine	Kharkiv Region, Zmiyiv District, Skrypayi village; pine forest					22,5	7,2	1,291,8867,47,2
CTAB	30	yes	no	*Veronica*	*pseudoorchidea*	*Pseudolysimachium*	OLD01227	Albach, D.C., Thielen, N., Mosyakin, S., Mosyakin, A. & Kuzemko, A.	1463b	OLD	HERBARIUM	2016-06-25	Ukraine	Oblast Kirowohrad: Ulianivka (Blagovischensk) District, near Kamiangyy Brid (village); meadow	48°14,831′ N	30°11,596′ E	130	2	21	35	1,281,7781,335
CTAB	30	yes	yes	*Veronica*	*pseudoorchidea*	*Pseudolysimachium*	OLD02899	Shiyan, N.M. and Zavyalova, L.V.	NSh: 001978, "1/2"	OLD	HERBARIUM	2013-06-26	Ukraine	Chernigiv Region, near town Ichnya, Ichnya National Park; damp meadow	50.837432' N	32.36892' E			21,6	41	1,721,9414141
CTAB	30	yes	yes	*Veronica*	*schmakovii*	*Pseudolysimachium*	OLD00666; AJUK 3321	Kamelin, R.V.; Shmakov, A.I.; Smirnov, S.; Kosachev, P.; Tichonov, D. & Antonjuk, E.	s.n.	OLD	HERBARIUM	1998-07-28	Russia	Republic Altai, Kosh-Agachskii district, Plateau Ukok, lower reaches river Kara-Bulak near frontier Ak-Alacha	49°16′ N	87°28′ E			17,5	8,1	0,821,72188,98,1
CTAB	30	yes	no	*Veronica*	*schmakovii*	*Pseudolysimachium*	OLD00659	Shmakov, A.I.; Dorofeev, V.I.; Kuzev, M.G.; Djachenko, S.A.; German, D.A.; Borodina-Grabovskaja, A.E.; Chubarov, I.N.; Kostjukov, S.A.; Usik, N. A. & Kosachev, P.A.	715 (individual I3)	OLD	HERBARIUM	2001-07-28	Mongolia	Bajan-Ulegejskii ajmak, Mongolskii Altai ridge, North-Western slopes of the peaks 3321	48°43,5′ N	88°05′ E		2	20,6	30	1,181,8179,230
CTAB	30	yes	yes	*Veronica*	*schmidtiana*	*Pseudolysimachium*	OLD00346	Albach, D.C.	1043	OLD	HERBARIUM	2008-04-16	Germany	cultivated in Botanical Garden, University of Mainz ex BG Tübingen (172/813), 2005					18,7	7,7	1,372,0548,57,7
CTAB	30	yes	no	*Veronica*	*sclerophylla*	*Pentasepalae*	OLD02911	Kuznetzova, G.	s.n. Dups.: 08/09	OLD	HERBARIUM	1958-05-23	Ukraine	Donetzk Reg., Novoazovskiy Distr., Nature Reserve "Kchomutovski Steppe"					18,5	11	1,491,8885,311
CTAB	30	yes	no	*Veronica*	*serpyllifolia*	*Beccabunga*	OLD01118	Raimaondo, F.M.; Jury, S.L.; Gebauer, R.; Charpin, A.; Brullo, S.; Hofmann, H.; Mastracci, M.; Lakusic, D.; Pérez Caro, E.; Minissale, P.; ...	1228	OLD	HERBARIUM	1990-06-05	Italy	Madonie: Piano Battaglia, calcareous soil	37°52′ N	14°00′ E	1550		17,9	12	0,831,65122,512
CTAB	30	no	no	*Veronica*	"*longifolia*" (*brevibractealis*) evtl. species nova	*Pseudolysimachium*	ALTB	V. Nikitin, V. Byalt, M. Azarkina, I. Illarionova	No.1309	ALTB	HERBARIUM	2003-07-06	Russia	Tuva Republic, Piy-Chemsky district, right bank of Bolshoi Enisey (Biy-Chem) river upstream the town Kyzyl. – Wet meadows on the riverbank	51°55′ n.lat	94°25′ e. long.			22,5	22	1,482,31144,122
CTAB	30	no	no	*Veronica*	*spicata* f. *falz-feiniana*	*Pseudolysimachium*		Tuzson, J.	s.n.	BP	SILICA	1912-07-13	Ukraine	Cherson: "Habitat in stepibus Tauricis prope Ascania-Nova"					20,4	8,8	1,011,75201,28,8
CTAB	30	no	no	*Veronica*	*bashkiriensis*	*Pseudolysimachium*	KW000058076	Kotov, M.	s.n.	KW	HERBARIUM	1942-07-28	Russia	Bashkir Autonomous Soviet Socialist Republic, Chirishmi, Tatar cemetery					20,4	16	1,871,99129,516
CTAB	30	no	no	*Veronica*	*borysthenica*	*Pseudolysimachium*	KW000058078	Ostapko, V.M.	s.n.	KW	HERBARIUM	1981-07-21	Ukraine	Dnipropetrovsk distr., Krivoy Rog, the Arroyo Chervona, steppe slope					22,9	6,3	1,662,0297,76,3
CTAB	30	no	no	*Veronica*	*cretacea*	*Pseudolysimachium*	KW000058080	Ostapko, V.M. and Maximova, S.A.	s.n.	KW	HERBARIUM	1987-06-28	Ukraine	prov. Lugansk, distr. Melovoje, pagus Streljtzovka, steppa petrophytica in rupite remanente cretacea					20,8	7,1	1,382,0951,97,1
CTAB	30	no	no	*Veronica*	*donetzica*	*Pseudolysimachium*	KW000058081	Ostapko, V.M.	s.n.	KW	HERBARIUM	1981-08-20	Ukraine	prov. Lugansk, distr. Sverdlovsk, pagus Provalje, locus Regiae rupes					22,4	3,26	1,132,0999,63,26
CTAB	30	no	no	*Veronica*	*gryniana*	*Pseudolysimachium*	KW000058083	Gryn, F. Dobrochaeva, D.	s.n.	KW	HERBARIUM	1939-06-17	Ukraine	Ukrainian SSR, Donetsk distr., Sloviansk reg. vil. Krasnopole, on the edge of forest					22,8	8,9	1,432,98117,98,9
CTAB	30	no	no	*Veronica*	*maeotica*	*Pseudolysimachium*	KW000058084	Grishko, T.	s.n.	KW	HERBARIUM	1952-06-03	Ukraine	RSS Ucr., dit. Donetzica, distr. Novoasovicus, p. Chomutovo, reservatum steppaceum Chomutovskaja stepj nominatum					20,7	11	1,662,07131,811
CTAB	30	no	no	*Veronica*	*paczoskiana*	*Pseudolysimachium*	OLD02918	Klokov, M.	s.n. Dups.: 07/07	OLD	HERBARIUM	1972-07-27	Russia	Cherkasy Reg., Kanivskyi district, village Mykchajlivka; Mykchajlivski pine forest					20,6	9,3	1,711,9787,69,3
CTAB	30	no	no	*Veronica*	*pontica*	*Pseudolysimachium*	KW000058099	Ostapko, V.M.	s.n.	KW	HERBARIUM	1987-07-03	Ukraine	prov. Lugansk, distr. Belovodsk, pagus Novolimarevka, steppa arenosa supra delivibus cretaceis					21,4	10	1,792,07129,210
CTAB	30	no	no	*Veronica*	*semiglabrata*	*Pseudolysimachium*	KW000058109	Ostapko, V.M.	s.n.	KW	HERBARIUM	1987-07-03	Ukraine	prov. Lugansk, op. Markovka, declive stepposum					20,7	8,7	1,472,0978,78,7
CTAB	30	no	no	*Veronica*	*sergievskiana*	*Pseudolysimachium*	OLD		s.n.	OLD	HERBARIUM	2017-08-13	Russia	Zabaykalskiy kray, ?					14,5	15	1,542,0188,215
CTAB	30	no	no	*Veronica*	"*tvelevii*" (maybe: *tzvelevii*)	*Pseudolysimachium*	KW093798			KW		2009-07-05							22	19	1,182,1888,719
CTAB	30	no	no	*Veronica*	*gryniana*	*Pseudolysimachium*	KW081622			KW		2006-06-24							21,1	3,61	1,372,2283,53,61
CTAB	30	no	no	*Veronica*	*hololeuca*	*Pseudolysimachium*	KW00104841			KW		2012-07-19							20,6	9,9	1,162,0765,39,9
CTAB	30	no	no	*Veronica*	*maeotica*	*Pseudolysimachium*	KW014286			KW		1990-07-01							20,6	6,6	1,632,041006,6
CTAB	30	no	no	*Veronica*	*steppacea*	*Pseudolysimachium*	KW009164			KW		2002-07-01							21	8,3	1,612,1488,58,3
CTAB	30	no	no	*Veronica*	*steppacea*	*Pseudolysimachium*	KW042384			KW		2004-??-16							21	8,1	1,22,1763,18,1
CTAB	30	no	no	*Veronica*	*steppacea*	*Pseudolysimachium*	KW009165			KW		2002-07-01							21,3	16	1,422,0859,216
CTAB	30	no	no	*Veronica*	*viscosula*	*Pseudolysimachium*	KW058848			KW		1974-06-19							21,4	7,5	1,972143,37,5
CTAB	30	no	no	*Veronica*	*viscosula*	*Pseudolysimachium*	KW027632			KW		1990-06-27							22,5	9,1	1,442,08115,89,1
Column	40	yes	no	*Veronica*	*barrelieri* subsp. *andrasovszkyi*	*Pseudolysimachium*	BP757299	Barina et al.	21604	BP	SILICA	2012-06-24	Albania	Diber, Komsi	41.57501N	19.94322E			10,1	9,66	2,3232,12992,64510,6
Column	40	yes	no	*Veronica*	*barrelieri* subsp. *andrasovszkyi*	*Pseudolysimachium*	BP761126	Barina & Pifko	22646	BP	SILICA	2012-06-23	Albania	Puke, Mt. Terbosh	42.00849N	19.91709E			9,6	4,45	2,3172,12763,5924,26
Column	40	yes	no	*Veronica*	*barrelieri* subsp. *andrasovszkyi*	*Pseudolysimachium*	BP768015	Barina et al.	23897	BP	SILICA	2012-06-24	Albania	Shkoder, south of Vau i Dejes Reservoir	42.05792N	19.68168E			10,2	4,31	2,2592,09361,0074,19
Column	40	yes	no	*Veronica*	*dahurica*	*Pseudolysimachium*	ALTB	Ebel A.L., Verhozina A.V., Mihaylova S.I., Strelnikova T.O., Sheremetova S.A., Hrustaleva I.A., Murashko V.V.	48448	ALTB	HERBARIUM	2017-08-17	Russia	Zabaykalskiy krai. Nerchinsko-Zavodskiy distr., near village Nerchinskiy-Zavod, riverbank Urov. Meadow steppe at the foot of the slope	N051°52'31.08"	E120°00'06.20"			9,3	5,09	2,2272,102110,7645,04
Column	40	yes	no	*Veronica*	*dahurica*	*Pseudolysimachium*	ALTB	Ebel A.L., Verhozina A.V., Mihaylova S.I., Strelnikova T.O., Sheremetova S.A., Hrustaleva I.A., Murashko V.V.	48450	ALTB	HERBARIUM	2017-08-13	Russia	Zabaykalskiy kray. Karyimskiy district, near village Zhimbira. Steppe slopes	N051°31'02.74"	E114°01'34.74'			7,4	12,3	2,2172,11689,44313,1
Column	40	yes	no	*Veronica*	*dahurica*	*Pseudolysimachium*	ALTB	Ebel A.L., Verhozina A.V., Mihaylova S.I., Strelnikova T.O., Sheremetova S.A., Hrustaleva I.A., Murashko V.V.	48453	ALTB	HERBARIUM	2017-08-16	Russia	Zabaykalskiy kray. Aleksandrovo-Zavodskiy district, 13 km south- east from village Aleksandrovskiy Zavod. Nerchinskiy mountain range. Left Bank of the river Malaya Borzya, South-West the slope of the mountain Murashiha. Grass-steppe meadow	N051°48'14.30"	E118°02'42.27"			9,5	7,68	2,3832,13251,4727,46
Column	40	yes	no	*Veronica*	*incana*	*Pseudolysimachium*	ALTB	G.V. Matyashenko	34171	ALTB	HERBARIUM	1977-08-08	Russia	Irkutskaya region, Olkhonskiy district, Olkhon island, Cape Kobyl'ja Golova (Chorin-Irgi). The North-Western slope	N053°03'44.00"	E106°54'14.00"			6,6	4,49	2,3572,07683,7644,26
Column	40	yes	no	*Veronica*	*incana*	*Pseudolysimachium*	ALTB	G.V. Matyashenko	36885	ALTB	HERBARIUM	1976-08-01	Russia	Irkutskaya oblast, Olkhonskiy district, lake Baikal, Bay Tashkay, South-East of village Tashkay. The top of the mountain, stone fields, steppe	N053°02'37.00"	E106°28'27.00"			5,8	8,9	2,2271,99223,3948,03
Column	40	yes	no	*Veronica*	*incana*	*Pseudolysimachium*	ALTB	L.N. Tyulina	43461	ALTB	HERBARIUM	1958-08-13	Russia	Irkutskaya oblast, Olkhonskiy district, lake Baikal, the North-Western coast of Baikal, Cape Pokoyniki. Pebble shore shaft at the lake	N054°00'31.00"	E108°12'58.00"			8,4	2,52	2,3962,06698,5782,29
Column	40	yes	no	*Veronica*	*incana*	*Pseudolysimachium*	ALTB	L.N. Tyulina	48524	ALTB	HERBARIUM	1958-08-02	Russia	Irkutskaya oblast, Olkhonskiy district, lake Baikal, the North-Western coast of Baikal, Cape Onguren. Rocky steppe slope to the lake	N054°00'31.00"	E108°12'58.00"			10,5	11	2,2921,95621,74510,4
Column	40	yes	no	*Veronica*	*incana*	*Pseudolysimachium*	ALTB	M.G. Azovsky	46249	ALTB	HERBARIUM	1987-07-07	Russia	Irkutskaya oblast, Olkhonskiy district, vicinities village Ozera. Meadow steppe	N052°42'00.14"	E106°21'57.90"			4,7	3,77	2,422,0848,8373,38
Column	40	yes	no	*Veronica*	*incana*	*Pseudolysimachium*	ALTB	S.G. Kazanovsky	48525	ALTB	HERBARIUM	1999-08-05	Russia	Irkutskaya oblast, Olkhonskiy district, lake Baikal, Malomorskoe coast, 1 km West of cape Ulibra. Stony steppe					6,3	4,81	2,4142,10678,4754,4
Column	40	yes	no	*Veronica*	*arenosa*	*Pseudolysimachium*	ALTB	S.A. Dyachenko.	No.1	ALTB	HERBARIUM	2005-06-24	Mongolia	Khobdo aimak, Mongolian Altai, NE macroslope of Arshantyn-Nuruu range, vicin. oft he spring Ulyastajn-Sala; Stony slopes, meadow steppes, Caragana & Artemisia communities.	46°22′ N	91°14′ E	1900		9,8	9,25	1,1942,03836,9838,82
Column	40	yes	no	*Veronica*	*arenosa*	*Pseudolysimachium*	ALTB	S.A. Dyachenko & P.A. Kosachev.	No.4	ALTB	HERBARIUM	2004-06-24	Mongolia	Khobdo distr., Mongolian Altai, Shadzgaityn-Nuruu range, N macroslope, valley of the left tributary of Ulyastajn-gol; meadow and steppe slopes	46°21′ N	91°41′ E			11,1	3,11	1,7472,09976,8362,98
Column	40	yes	no	*Veronica*	*arenosa*	*Pseudolysimachium*	ALTB	S.A. Dyachenko & P.A. Kosachev.	No.5	ALTB	HERBARIUM	2004-07-01	Mongolia	Khobdo distr., Mongolian Altai, middle reaches of Uenchijn-gol; stony slopes, limestone rocks	46°22′ N	92°07′ E			10,4	6,27	2,0632,09487,7086,1
Column	40	yes	no	*Veronica*	*laeta*	*Pseudolysimachium*	ALTB	Shmakov A. I., Ebel A. L. Kashcheev, M., Dyachenko S., Soloviev A.	No.2	ALTB	HERBARIUM	1991-07-21	Kazakhstan	Taldy-Kurganskaya oblast, Dzungarian Alatau, Kapal'skiy district, the upper river Sarkand, steppe slope of Western exposure					9,5	8,35	2,2652,04105,228,13
Column	40	yes	no	*Veronica*	*laeta*	*Pseudolysimachium*	ALTB	Shmakov A. I., Ebel A. L. Kashcheev, M., Dyachenko S., Soloviev A.	No.4	ALTB	HERBARIUM	1991-07-21	Kazakhstan	Taldy-Kurganskaya oblast, Dzungarian Alatau, Kapal'skiy district, the upper river Sarkand, steppe slope of Western exposure					9,2	23,8	2,1822,04791,14622,3
Column	40	yes	no	*Veronica*	*laeta*	*Pseudolysimachium*	ALTB	Shmakov A. I., Ebel A. L. Kashcheev, M., Dyachenko S., Soloviev A.	No.5	ALTB	HERBARIUM	1991-07-21	Kazakhstan	Taldy-Kurganskaya oblast, Dzungarian Alatau, Kapal'skiy district, the upper river Sarkand, steppe slope of Western exposure					10	15,2	2,1892,02468,18214,8
Column	40	yes	no	*Veronica*	*alatavica*	*Pseudolysimachium*	ALTB	Laz'kov G.A.	s.n.	ALTB	HERBARIUM	2004-08-10	Kirgizstan	Kungey Ala-too, river Chok-Aksu					10,1	11,8	2,0142,053142,5911,1
Column	40	yes	no	*Veronica*	*grisea*	*Pseudolysimachium*	ALTB	V. Byalt, V. Nikitin, I. Illarionova, V. Shaulo & al.	No. 415	ALTB	HERBARIUM	2003-07-01_02	Russia	Tuva, Dzun-Khemchiksky distr., road Kyzyl-Shagonar, rv. Shemi. Sands and pebbles on banks	51°16′ N	91°11′ E	766		8,5	16,8	2,122,06930,07716,4
Column	40	yes	yes	*Veronica*	*altaica*	*Pseudolysimachium*	OLD00641	Kamelin, R.V.; Shmakov, A.I.; Dorofeev, V.I.; Smirnov, S.V.; Chubarov, I.; Antonjuk, E.; Kosachev, P. & Kuzev, M.G.	s.n.	OLD	HERBARIUM	2000-07-02	Russia	Altaiskii krai, Krasnoschekovskii district, vicinity village Kharlovo, right bank river Charysh	51°41′ N	82°49′ E			9,8	1,91	2,2652,07430,691,88
Column	40	yes	yes	*Veronica*	*barrelieri* (likely: *steppacea*)	*Pseudolysimachium*	OLD02924	Stepura (?)	126539	OLD	HERBARIUM	1962-06-15	Ukraine	Donetsk Region, Novoazovskiy District, Khomutovsty Steppe Nature Reserve					10,7	2,26	1,9682,09417,8332,12
Column	40	yes	yes	*Veronica*	*chamaedrys*	*Chamaedrys*	OLD00661	Buldakova; Paschenko	s.n.	OLD	HERBARIUM	1985-07-03	Russia	Altaiskii krai, Kurjinskii district, vicinity village 8 Marta, lake Beloe					6,6	1,55	2,3271,997145,8311,28
Column	40	yes	no	*Veronica*	*chamaedrys*	*Chamaedrys*	OLD00662	Buldakova; Paschenko	s.n.	OLD	HERBARIUM	1985-07-04	Russia	Altaiskii krai, Kurjinskii district, mountain Sinjucha; mixed forest					3,5	29	2,0741,96230,62526,1
Column	40	yes	no	*Veronica*	*chamaedrys*	*Chamaedrys*	OLD00663	Tamilova	s.n.	OLD	HERBARIUM	1987-06-22	Russia	Altaiskii krai, vicinity of station Povaliha; marge					5,4	4,75	2,0661,88140,5674,3
Column	40	yes	no	*Veronica*	*chamaedrys* subsp. *chamaedrys*	*Chamaedrys*	OLD00524	Mikoláš, V.	7083	OLD	HERBARIUM	1992-08-06	Slovakia	Slovakia centralis, Západné Tatry Mts., Zuberec village, Javorina Mt. (S of Osobitá Mt.)	49°15'23"N	19°43'23"E	1530		10,3	13,4	1,8951,9264,01212,3
Column	40	yes	no	*Veronica*	*chamaedrys* var. *Villosa* Mikoláš ined.	*Chamaedrys*	OLD01107	Mikoláš, V.	16018	OLD	HERBARIUM	1986-05-01	Slovakia	Slovakia orientalis, Košice, Botanical Garden of University of P.J.Šafárika, at terraces	48°44'07"N	21°14'10"E	280		5,6	19,1	2,31,98683,77817,7
Column	40	yes	no	*Veronica*	*densiflora*	*Stenocarpon*	OLD00664	Kamelin, R.V.; Shmakov, A.I.; Dorofeev, V.I.; Goljakov, P.; Solovjev, A.; Kascheev, M. Luchkin, E. & Smirnov, S.	s.n.	OLD	HERBARIUM	1995-07-06	Russia	Republic Altai, Kosh-Agachskii district, Chikhacheva ridge, pass Bugusun; alpine meadows	50°11′ N	89°25′ E			5,7	15,7	2,3311,99433,20814,3
Column	40	yes	yes	*Veronica*	*filifolia*	*Pentasepalae*	OLD02902	Kotov, M. and Omeltchuk, T.	s.n. Dups.: 05/10	OLD	HERBARIUM	1958-05-28	Ukraine	West Transcaucasia, Novorosijsk, Range Markotch; on the limestone					10,3	6,84	1,711,94814,476,24
Column	40	yes	yes	*Veronica*	*incana*	*Pseudolysimachium*	OLD02906	Klokov, M.	126570 Dups.: 05/10	OLD	HERBARIUM	1973-07-30	Ukraine	Kyiv Reg., station Klavdiievo; Pine-wideleaf forests					9,3	0,96	2,3962,00542,630,863
Column	40	yes	no	*Veronica*	*incana*	*Pseudolysimachium*	OLD02907	Klokov, M. and Dubovik, O.	s.n. Dups.: 03/07	OLD	HERBARIUM	1973-07-18	Ukraine	Kyiv Reg., Irpin'; Pine-wideleaf forestes the other side of the river Irpin					10,2	NA	2,0611,87677,253NA
Column	40	yes	no	*Veronica*	*incana*	*Pseudolysimachium*	OLD02909	Tkatchenko, V.	s.n. Dups.: 07/07	OLD	HERBARIUM	1963-07-04	Ukraine	Lugansk Reg., Stanichno-Luganske; Steppe slopes on the right shore of the Severskyi Donetz River					10,4	23,2	2,0191,98552,32622
Column	40	yes	no	*Veronica*	*kiusiana*	*Pseudolysimachium*	OLD00365	Albach, D.C.	1060	OLD	HERBARIUM	2008-06-02	Korea	Botanischer Garten Mainz; cultivated from seeds ex Botanical Garden Yeomiji, Korea					11,1	4,58	2,0822,06986,554,23
Column	40	yes	no	*Veronica*	*kiusiana* var. *maxima*	*Pseudolysimachium*	OLD00213	Albach, D.C.	1040	OLD	HERBARIUM	2007-05-29	Germany	cultivated at Botanical Garden Mainz, ex BG Bonn, XX-0-Bonn-22013				2	10,5	7,29	1,0711,7827,4647,17
Column	40	yes	no	*Veronica*	*linariifolia*	*Pseudolysimachium*	OLD00370	Albach, D.C. & Zhao, L.	1214	OLD	HERBARIUM	2011-07-17	China	Shaanxi prov., Ningshan County; outside Jiankou; open vegetation next to fields	33.653N	108.646E	800		8,8	6,84	2,252,09234,2126,52
Column	40	yes	yes	*Veronica*	*maeotica*	*Pseudolysimachium*	OLD01194	Albach, D.C., Thielen, N., Mosyakin, S., Mosyakin, A.	1486	OLD	HERBARIUM	2016-07-01	Ukraine	Oblast Kiew: Koncha Zaspa meadows; clearing in pine forest margin, mossy	50°19,796′ N	30°34,064′ E	102	2	5,7	1,96	2,3482,118113,261,85
Column	40	yes	no	*Veronica*	*paczoskiana*	*Pseudolysimachium*	OLD02915	Klokov, M. and Zoz, I.	s.n. Dups.: 07/07	OLD	HERBARIUM	1976-07-28	Ukraine	Kcharkiv Reg., Zmiivskyi Distr., villae Skrypaji; Pine forest					10,2	11,2	1,3911,79214,55810,5
Column	40	yes	no	*Veronica*	*paczoskiana*	*Pseudolysimachium*	OLD02916	Klokov, M. and Dubovik, O.	s.n. Dups.: 03/38	OLD	HERBARIUM	1973-07-20	Ukraine	Station Maljutinka near Kyiv sity; Pine forest					11,8	2,75	2,0852,02256,4482,48
Column	40	yes	no	*Veronica*	*paczoskiana*	*Pseudolysimachium*	OLD02919	Klokov, M. and Dubovik, O.	s.n. Dups.: 28/38	OLD	HERBARIUM	1973-07-20	Ukraine	Station Maljutinka near Kyiv sity; Pine forest					10,4	4,77	2,172,02194,7524,45
Column	40	yes	no	*Veronica*	*paczoskiana*	*Pseudolysimachium*	OLD02923	Klokov, M. and Dubovyk, O.	126441 Dups.: 38/38	OLD	HERBARIUM	1973-07-20	Ukraine	near Kyiv (Kiev), Malyutynka railway station; Pine forest					9	8,37	2,1571,95836,7667,84
Column	40	yes	yes	*Veronica*	*paczoskiana*	*Pseudolysimachium*	OLD02925	Klokov, M. and Zoz, I.	126428	OLD	HERBARIUM	1976-06-25	Ukraine	Kharkiv Region, Zmiyiv District, Skrypayi village; pine forest					11,2	4,48	2,3322,0740,9624,26
Column	40	yes	no	*Veronica*	*pseudoorchidea*	*Pseudolysimachium*	OLD01227	Albach, D.C., Thielen, N., Mosyakin, S., Mosyakin, A. & Kuzemko, A.	1463b	OLD	HERBARIUM	2016-06-25	Ukraine	Oblast Kirowohrad: Ulianivka (Blagovischensk) District, near Kamiangyy Brid (village); meadow	48°14,831′ N	30°11,596′ E	130	2	9,3	3,4	2,3732,08227,673,34
Column	40	yes	yes	*Veronica*	*pseudoorchidea*	*Pseudolysimachium*	OLD02899	Shiyan, N.M. and Zavyalova, L.V.	NSh: 001978, "1/2"	OLD	HERBARIUM	2013-06-26	Ukraine	Chernigiv Region, near town Ichnya, Ichnya National Park; damp meadow	50.837432' N	32.36892' E			9	3,43	2,3072,07889,4873,4
Column	40	yes	yes	*Veronica*	*schmakovii*	*Pseudolysimachium*	OLD00666; AJUK 3321	Kamelin, R.V.; Shmakov, A.I.; Smirnov, S.; Kosachev, P.; Tichonov, D. & Antonjuk, E.	s.n.	OLD	HERBARIUM	1998-07-28	Russia	Republic Altai, Kosh-Agachskii district, Plateau Ukok, lower reaches river Kara-Bulak near frontier Ak-Alacha	49°16′ N	87°28′ E			9,8	9,87	2,0991,98916,7119,61
Column	40	yes	no	*Veronica*	*schmakovii*	*Pseudolysimachium*	OLD00659	Shmakov, A.I.; Dorofeev, V.I.; Kuzev, M.G.; Djachenko, S.A.; German, D.A.; Borodina-Grabovskaja, A.E.; Chubarov, I.N.; Kostjukov, S.A.; Usik, N. A. & Kosachev, P.A.	715 (individual I3)	OLD	HERBARIUM	2001-07-28	Mongolia	Bajan-Ulegejskii ajmak, Mongolskii Altai ridge, North-Western slopes of the peaks 3321	48°43,5′ N	88°05′ E		2	9,5	1,76	2,1972,03159,2011,66
Column	40	yes	yes	*Veronica*	*schmidtiana*	*Pseudolysimachium*	OLD00346	Albach, D.C.	1043	OLD	HERBARIUM	2008-04-16	Germany	cultivated in Botanical Garden, University of Mainz ex BG Tübingen (172/813), 2005					9,8	10,4	2,4032,12200,07710,1
Column	40	yes	no	*Veronica*	*sclerophylla*	*Pentasepalae*	OLD02911	Kuznetzova, G.	s.n. Dups.: 08/09	OLD	HERBARIUM	1958-05-23	Ukraine	Donetzk Reg., Novoazovskiy Distr., Nature Reserve "Kchomutovski Steppe"					7,7	20,4	1,7651,7727,34121,8
Column	40	yes	no	*Veronica*	*serpyllifolia*	*Beccabunga*	OLD01118	Raimaondo, F.M.; Jury, S.L.; Gebauer, R.; Charpin, A.; Brullo, S.; Hofmann, H.; Mastracci, M.; Lakusic, D.; Pérez Caro, E.; Minissale, P.; ...	1228	OLD	HERBARIUM	1990-06-05	Italy	Madonie: Piano Battaglia, calcareous soil	37°52′ N	14°00′ E	1550		7,9	9,67	2,0731,99161,6148,94
Incubation time 2h	120	no	yes	*Veronica*	*altaica*	*Pseudolysimachium*	OLD00641	Kamelin, R.V.; Shmakov, A.I.; Dorofeev, V.I.; Smirnov, S.V.; Chubarov, I.; Antonjuk, E.; Kosachev, P. & Kuzev, M.G.	s.n.	OLD	HERBARIUM	2000-07-02	Russia	Altaiskii krai, Krasnoschekovskii district, vicinity village Kharlovo, right bank river Charysh	51°41′ N	82°49′ E			10,6	4,72	0,7611,7557,0544,73
Incubation time 2h	120	no	yes	*Veronica*	*barrelieri* (likely: *steppacea*)	*Pseudolysimachium*	OLD02924	Stepura (?)	126539	OLD	HERBARIUM	1962-06-15	Ukraine	Donetsk Region, Novoazovskiy District, Khomutovsty Steppe Nature Reserve					9,8	1,11	0,9741,84123,7521,1
Incubation time 2h	120	no	yes	*Veronica*	*chamaedrys*	*Chamaedrys*	OLD00661	Buldakova; Paschenko	s.n.	OLD	HERBARIUM	1985-07-03	Russia	Altaiskii krai, Kurjinskii district, vicinity village 8 Marta, lake Beloe					7,1	1,26	1,5991,75112,9971,19
Incubation time 2h	120	no	yes	*Veronica*	*filifolia*	*Pentasepalae*	OLD02902	Kotov, M. and Omeltchuk, T.	s.n. Dups.: 05/10	OLD	HERBARIUM	1958-05-28	Ukraine	West Transcaucasia, Novorosijsk, Range Markotch; on the limestone					10,1	1,03	0,8671,83922,2691,02
Incubation time 2h	120	no	yes	*Veronica*	*incana*	*Pseudolysimachium*	OLD02906	Klokov, M.	126570 Dups.: 05/10	OLD	HERBARIUM	1973-07-30	Ukraine	Kyiv Reg., station Klavdiievo; Pine-broadleaf forests					10,9	2,2	1,0721,76218,8212,23
Incubation time 2h	120	no	yes	*Veronica*	*maeotica*	*Pseudolysimachium*	OLD01194	Albach, D.C., Thielen, N., Mosyakin, S., Mosyakin, A.	1486	OLD	HERBARIUM	2016-07-01	Ukraine	Oblast Kiew: Koncha zaspa meadows; clearing in pine forest margin, mossy	50°19,796′ N	30°34,064′ E	102	2	9,3	6,17	1,8361,85419,6066,09
Incubation time 2h	120	no	yes	*Veronica*	*paczoskiana*	*Pseudolysimachium*	OLD02925	Klokov, M. and Zoz, I.	126428	OLD	HERBARIUM	1976-06-25	Ukraine	Kharkiv Region, Zmiyiv District, Skrypayi village; pine forest					10,6	0,115	0,4251,3965,1470,114
Incubation time 2h	120	no	yes	*Veronica*	*pseudoorchidea*	*Pseudolysimachium*	OLD02899	Shiyan, N.M. and Zavyalova, L.V.	NSh: 001978, "1/2"	OLD	HERBARIUM	2013-06-26	Ukraine	Chernigiv Region, near town Ichnya, Ichnya National Park; damp meadow	50.837432' N	32.36892' E			10,9	3,63	1,8011,87731,1013,69
Incubation time 2h	120	no	yes	*Veronica*	*schmakovii*	*Pseudolysimachium*	OLD00666; AJUK 3321	Kamelin, R.V.; Shmakov, A.I.; Smirnov, S.; Kosachev, P.; Tichonov, D. & Antonjuk, E.	s.n.	OLD	HERBARIUM	1998-07-28	Russia	Republic Altai, Kosh-Agachskii district, Plateau Ukok, lower reaches river Kara-Bulak near frontier Ak-Alacha	49°16′ N	87°28′ E			8,7	3,09	1,7711,94846,3692,91
Incubation time 2h	120	no	yes	*Veronica*	*schmidtiana*	*Pseudolysimachium*	OLD00346	Albach, D.C.	1043	OLD	HERBARIUM	2008-04-16	Germany	cultivated in Botanical Garden, University of Mainz ex BG Tübingen (172/813), 2005					9,8	0,829	1,1871,90915,5050,808
Incubation time 4h	240	no	yes	*Veronica*	*altaica*	*Pseudolysimachium*	OLD00641	Kamelin, R.V.; Shmakov, A.I.; Dorofeev, V.I.; Smirnov, S.V.; Chubarov, I.; Antonjuk, E.; Kosachev, P. & Kuzev, M.G.	s.n.	OLD	HERBARIUM	2000-07-02	Russia	Altaiskii krai, Krasnoschekovskii district, vicinity village Kharlovo, right bank river Charysh	51°41′ N	82°49′ E			7,4	1,92	1,2282,02924,2131,9
Incubation time 4h	240	no	yes	*Veronica*	*barrelieri* (likely: *steppacea*)	*Pseudolysimachium*	OLD02924	Stepura (?)	126539	OLD	HERBARIUM	1962-06-15	Ukraine	Donetsk Region, Novoazovskiy District, Khomutovsty Steppe Nature Reserve					10,7	3,24	1,6431,86733,7783,22
Incubation time 4h	240	no	yes	*Veronica*	*chamaedrys*	*Chamaedrys*	OLD00661	Buldakova; Paschenko	s.n.	OLD	HERBARIUM	1985-07-03	Russia	Altaiskii krai, Kurjinskii district, vicinity village 8 Marta, lake Beloe					6,3	2,28	0,9352,02725,9542,25
Incubation time 4h	240	no	yes	*Veronica*	*filifolia*	*Pentasepalae*	OLD02902	Kotov, M. and Omeltchuk, T.	s.n. Dups.: 05/10	OLD	HERBARIUM	1958-05-28	Ukraine	West Transcaucasia, Novorosijsk, Range Markotch; on the limestone					9,3	0,896	0,7481,8418,5030,885
Incubation time 4h	240	no	yes	*Veronica*	*incana*	*Pseudolysimachium*	OLD02906	Klokov, M.	126570 Dups.: 05/10	OLD	HERBARIUM	1973-07-30	Ukraine	Kyiv Reg., station Klavdiievo; Pine-broadleaf forests					10,9	5,26	1,191,89753,5615,23
Incubation time 4h	240	no	yes	*Veronica*	*maeotica*	*Pseudolysimachium*	OLD01194	Albach, D.C., Thielen, N., Mosyakin, S., Mosyakin, A.	1486	OLD	HERBARIUM	2016-07-01	Ukraine	Oblast Kiew: Koncha Zaspa meadows; clearing in pine forest margin, mossy	50°19,796′ N	30°34,064′ E	102	2	10,5	3,55	1,8931,97830,4073,52
Incubation time 4h	240	no	yes	*Veronica*	*paczoskiana*	*Pseudolysimachium*	OLD02925	Klokov, M. and Zoz, I.	126428	OLD	HERBARIUM	1976-06-25	Ukraine	Kharkiv Region, Zmiyiv District, Skrypayi village; pine forest					9,5	0,613	0,5291,77132,0740,605
Incubation time 4h	240	no	yes	*Veronica*	*pseudoorchidea*	*Pseudolysimachium*	OLD02899	Shiyan, N.M. and Zavyalova, L.V.	NSh: 001978, "1/2"	OLD	HERBARIUM	2013-06-26	Ukraine	Chernigiv Region, near town Ichnya, Ichnya National Park; damp meadow	50.837432' N	32.36892' E			8,5	5,92	1,0341,92944,7115,82
Incubation time 4h	240	no	yes	*Veronica*	*schmakovii*	*Pseudolysimachium*	OLD00666; AJUK 3321	Kamelin, R.V.; Shmakov, A.I.; Smirnov, S.; Kosachev, P.; Tichonov, D. & Antonjuk, E.	s.n.	OLD	HERBARIUM	1998-07-28	Russia	Republic Altai, Kosh-Agachskii district, Plateau Ukok, lower reaches river Kara-Bulak near frontier Ak-Alacha	49°16′ N	87°28′ E			7,5	3,4	1,3151,95250,393,37
Incubation time 4h	240	no	yes	*Veronica*	*schmidtiana*	*Pseudolysimachium*	OLD00346	Albach, D.C.	1043	OLD	HERBARIUM	2008-04-16	Germany	cultivated in Botanical Garden, University of Mainz ex BG Tübingen (172/813), 2005					8,7	2,68	1,0161,85923,0682,63