

TAXONOMIC STATUS OF *GALEOBDOLON LUTEUM* HUDS. (LAMIACEAE) FROM CLASSICAL TAXONOMY AND PHYLOGENETICS PERSPECTIVES

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This paper is both a review and a study. It discusses the taxonomic status of Yellow Archangel (*Galeobdolon luteum* Huds.) from historical and contemporary perspectives, and gives a comprehensive list of synonyms for the discussed genera, species and lower taxonomic units, including their publication details. In the study it is postulated that *G. luteum* should be included in the genus *Lamium*. The hypothesis is verified by a comparative analysis between the representatives of the genera *Galeobdolon* and *Lamium* in four DNA regions: ITS, *accD*, *rpoC1* and *trnH-psbA*. The analysis supported the determination of phylogenetic relationships among the studied taxa: *G. luteum* is not genetically distant enough from *Lamium* to be considered a separate genus, and integration of *Galeobdolon* and *Lamium* is legitimate.

Key words: *Galeobdolon luteum*, taxonomy, phylogeny, *accD*, ITS, *rpoC1*, *trnH-psbA*, Lamiaceae.

INTRODUCTION

Plant taxonomy, one of the oldest biological disciplines, is a relevant and important science (Sivarajan and Robson, 1991) which continues to develop. Taxonomic methods have undergone considerable change through the centuries. In the past, taxonomy was based mainly on plant morphology and anatomy; experimental results play a more important role in contemporary research (Stace, 1991). The horizons of taxonomy have considerably expanded over the years. The role of taxonomists is no longer reduced to that of plant identifiers. Contemporary taxonomy also deals with the origin and evolution of variation in organisms, the breeding behavior of populations, and structural and functional details (Sivarajan and Robson, 1991). Recent advances in taxonomy point to the need for continued updating of the plant classification system to maximize its conformity with plant phylogeny.

Changes in systematics inevitably lead to changes in nomenclature, resulting in the presence of synonymous names in the scientific literature. One of the many species subject to taxonomic debate is *Galeobdolon luteum* (Yellow Archangel). It is a fairly widespread taxon which occurs naturally in most

parts of Europe and the Caucasian region of Asia (Ball, 1972; Hulten and Fries, 1986) and is frequently described in botanical literature. Researchers continue to argue its taxonomic status, which is why the species appears under a variety of names.

This paper discusses the taxonomic status of *G. luteum*. We broadly review the literature to set out the research problem and the approaches of different taxonomists. We also present the results of our own research aimed at verifying a proposal to include *G. luteum* in the genus *Lamium* as a species of dead-nettle. To test it we made a comparative analysis of representatives of the genera *Galeobdolon* and *Lamium*. The results supported our determination of the phylogenetic relationships among the studied taxa.

HISTORY OF YELLOW ARCHANGEL'S NOMENCLATURE

Yellow Archangel is described under a variety of synonymous names due to the long history of its nomenclature and frequent changes in taxonomic approaches over the centuries. The controversy over the most appropriate name begins at the generic

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TABLE 1. Synonyms for the genus *Galeobdolon* given in chronological order

Generic name	Publication
<i>Galeobdolon</i> Dillenius	Cat. Pl., App.: 103 (1719)
<i>Lamiastrum</i> Heister ex Fabricius	Enum. Meth. Pl. Hort. Med. Helmstad.: 51 (1759)
<i>Galeobdolon</i> Adanson	Fam. Pl. 2: 190, 560 (1763)
<i>Galeobdolon</i> Hudson	Fl. Angl., ed. 2, 257, (1778)
<i>Pol(1)ichia</i> Schrank	Acta Acad. Mogunt. Erfurt: 35 (1781)
<i>Lamium</i> sect. <i>Galeobdolon</i> (Hudson) Bentham	DC. Prodr. 12: 511 (1848)
<i>Lamium</i> subgen. <i>Galeobdolon</i> (Hudson) Aschers	Fl. Brandenb. 1: 525 (1864).

TABLE 2. Synonyms for *G. luteum* s. str. given in alphabetical order

Taxon	Publication
<i>Galeobdolon galeobdolon</i> (L.) H.Karst.	III. Repet. Pharm.-Med. Bot. 1010 (1886)
<i>Galeobdolon galeopsis</i> Curt.	Fl. Lond. 2: t. 223 (1798)
<i>Galeobdolon luteum</i> Huds.	Fl. Angl., ed. 2, 1:258 (1778)
<i>Galeobdolon luteum</i> var. <i>tatrae</i> Ullepitsch	Österr. Bot. Z. 37:84 85 (1887)
<i>Galeobdolon umbrosum</i> Wibel	Prim. Fl. Werth.: 139 (1799)
<i>Galeobdolon vulgare</i> (Pers.) Pers.	Syn. Pl. 2:122 (1807)
<i>Galeopsis galeobdolon</i> L.	Spec. Pl. : 519 (1753)
<i>Lamiastrum galeobdolon</i> subsp. <i>galeobdolon</i> (L.) Ehrend. et Polatschek	Österr. Bot. Z. 113: 108 (1966)
<i>Lamium galeobdolon</i> (L.) Crantz	Strip. Austr. Fasc. ed. 2, 4: 262 (1763)
<i>Lamium galeobdolon</i> (L.) Nath.	Fl. Monsp.: 19 (1756)
<i>Lamium galeobdolon</i> (L.) L.	Amoen. Acad. 4: 485 (1759)
<i>Lamium galeobdolon</i> subsp. <i>vulgare</i> (Pers.) Hayek	Rep. Spec. Beih. 30 (2): 272 (1929)
<i>Lamium galeobdolon</i> var. <i>vulgare</i> (Pers.) Briq.	Lab. Alp. Marit. : 319 (1893)
<i>Lamium luteum</i> (Huds.) Krock.	Suppl. Fl. Siles. 2:148 (1823)
<i>Lamium vulgare</i> (Pers.) Fritsch	Exk. Fl.: 472 (1897)
<i>Leonurus galeobdolon</i> (L.) Scop.	Fl. Carn. no. 705 (1760)
<i>Polichia galeobdolon</i> (L.) Willd.	Fl. Berol. Prodr. 198 (1787)
<i>Pollichia galeobdolon</i> (L.) Schrank	Act. Erford. 3: 35 (1782)
<i>Pol(l)ichia vulgaris</i> Pers.	Usteri, Ann. Bot. 14: 39 (1795)

level. The rule of priority is a necessary consideration. The very first name of the genus, proposed by Dillenius in 1719, was *Galeobdolon* (Tab. 1). Formulated before the publication of Linnaeus's (1753) *Species Plantarum*, it is not officially recognized. It belongs to the category of "pre-Linnaean" names and shall not be included in the naming process based on the provisions of the International Code of Botanical Nomenclature (ICBN) (McNeill, 2006). The first officially published name of Yellow Archangel was *Lamiastrum* Heister ex Fabr. which appeared in the scientific literature in 1759 (Tab. 1). In 1763, *Galeobdolon* was used repeatedly by Adanson. In light of an earlier publication by Fabricius (1759), that name probably falls into the *nomen superfluum* category. These points indicate

that the use of the name *Lamiastrum* is more justified. The term *Galeobdolon*, found at the rank of section or subgenus (Tab. 1), is often used by researchers and commonly encountered in the literature. Yellow Archangel was not always recognized as a separate genus. In earlier studies it appeared as a species of the genus *Pol(l)ichia* or, more frequently, it was classified as a member of the genus *Lamium* under the name *L. galeobdolon*. Based on the findings in a recent Polish checklist (Mirek et al., 2002), the name *Galeobdolon luteum* will be used in this study.

The multitude of generic names means an even greater number of species synonyms. Those synonyms are listed alphabetically in Table 2. In addition to nominative subspecies, three other

TABLE 3. Synonyms for subspecies of *G. luteum* given in alphabetical order

Taxon	Publication
subsp. <i>argentatum</i>	
<i>Galeobdolon argentatum</i> Smejkal	Preslia 47: 243 (1975)
<i>Lamiastrum argentatum</i> (Smejkal) Soják	Zprávy Krajsk. Vlastiv. Muz. Olomouci 215: 4 (1982)
<i>Lamiastrum galeobdolon</i> subsp. <i>argentatum</i> (Smejkal) Stace	Watsonia 17: 442 (1989)
<i>Lamium argentatum</i> (Smejkal) Henker ex G.H.Loos	Florist. Rundbr. 31(1): 43 (1997).
<i>Lamium galeobdolon</i> f. <i>argentatum</i> (Smejkal) Mennema	Leiden Bot. Ser. 11: 46 (1989).
<i>Lamium galeobdolon</i> subsp. <i>argentatum</i> (Smejkal) J.Duvign	Bull. Jard. Bot. Natl. Belg. 57: 459 (1987).
subsp. <i>flavidum</i>	
<i>Galeobdolon flavidum</i> (F.Herm.) Holub	Folia Geobot. Phytotax. 5: 80 (1970)
<i>Lamiastrum flavidum</i> (F.Herm.) Ehrend.	Österr. Bot. Z. 122: 266 (1973)
<i>Lamiastrum galeobdolon</i> subsp. <i>flavidum</i> (Herm.) Ehrend. et Polatschek	Österr. Bot. Z. 113: 108 (1966)
<i>Lamium flavidum</i> Herm.	Ber. Bayer. Bot. Ges. 32: 145-146 (1958).
<i>Lamium galeobdolon</i> subsp. <i>flavidum</i> (Herm.) A. et D. Löve	Bot. Not. 114:55 (1961)
<i>Lamium pallidum</i> Herm.	Fl. Nord-Mitteleuropa: 864 (1956)
<i>Lamium galeobdolon</i> subsp. <i>pallidum</i> Herm. ex Rothm.	Exkursionsfl. Deutschl., Krit. Ergbd.: 266 (1963)
subsp. <i>montanum</i>	
<i>Cardiaca silvatica</i> Lam.	Fl. Fr. 2: 384 (1778)
<i>Galeobdolon endtmannii</i> (G.H.Loos) Holub	Preslia 70: 104 (1998)
<i>Galeobdolon luteum</i> Huds.	Fl. Angl. ed. 2, 1: 258 (1798)
<i>Galeobdolon luteum</i> subsp. <i>montanum</i> (Pers.) R.R.Mill	Fl. Turkey & E. Aegean Is. 7: 150 (1982)
<i>Galeobdolon luteum</i> var. <i>florentinum</i> Silva Tar.	Freiland-Staud. 105 (1910)
<i>Galeobdolon luteum</i> var. <i>montanum</i> (Pers.) Nyman	Consp. Fl. Eur. 576 (1881)
<i>Galeobdolon montanum</i> (Pers.) Pers. in Rchb.	Fl. Germ. Exc.: 860 (1832)
<i>Galeobdolon vulgare</i> var. <i>montanum</i> (Pers.) Pers.	Syn. Pl. 2:122 (1807)
<i>Lamiastrum galeobdolon</i> subsp. <i>montanum</i> (Pers.) Ehrend. et Polatschek	Österr. Bot. Z. 113: 108 (1966)
<i>Lamiastrum montanum</i> (Pers.) Ehrend.	Österr. Bot. Z. 122: 266 (1973)
<i>Lamium endtmannii</i> G.H.Loos	Florist. Rundbr. 31(1): 43 (1997).
<i>Lamium galeobdolon</i> subsp. <i>montanum</i> (Pers.) Hayek	Rep. Spec. Beih. 30 (2): 272 (1929)
<i>Lamium galeobdolon</i> var. <i>montanum</i> (Pers.) Pers.	Syn. Pl. 2: 122 (1807)
<i>Lamium montanum</i> (Pers.) Hoffm. ex Kabath	Fl. Gleiwitz 130 (1846)
<i>Lamium montanum</i> var. <i>florentinum</i> (Silva Tar.) Buttler & Schippm.	Bot. Naturschutz Hessen 6: 6 (1993)
<i>Lamium montanum</i> subsp. <i>endtmannii</i> (G.H.Loos) G.H.Loos	Jahrb. Bochum. Bot. Vereins 1: 124 (2010)
<i>Pollichia montana</i> Pers.	Usteri Ann. Bot. 14: 39 (1795)

subspecies are generally distinguished within *G. luteum*: *argentatum*, *flavidum* and *montanum*. Subspecies *montanum* was first described in the late 18th century (originally erroneously identified as a species of the genus *Cardiaca*). Subspecies *argentatum* and *flavidum* were defined as separate taxa only in the 20th century. An alphabetical list of synonyms under which the discussed subspecies appear in the literature is given in Table 3.

CONTEMPORARY TAXONOMIC APPROACH

There is still no consensus about the taxonomic rank and consequently the correct name of Yellow Archangel. Taxonomists have taken two opposing approaches. The first places Yellow Archangel in its own genus. Some taxonomists apply the genus name *Lamiastrum* (Polatschek, 1966; Ball, 1972; Rutkowski, 2011; Sheen et al., 2010), consistent

with ICBN principles (McNeill et al., 2006). However, *Galeobdolon* is still used as a generic name in many significant publications from Central Europe (Sychowa, 1967; Gladokova, 1978; Szafer et al., 1986; Dvořáková, 2000; Mirek et al., 2002; Rothmaler, 2007). The second approach includes Yellow Archangel in the genus *Lamium* as a species of dead-nettle (Mennema, 1989; Rosenbaumová et al., 2004; Castroviejo et al., 2010; Czarna and Bednorz, 2011; Govaerts et al., 2010).

A detailed taxonomic analysis of Yellow Archangel focuses on its affiliation to the genus and the choice of the correct name, but also involves identification of lower taxonomic units. As also in many historical studies, contemporary researchers generally distinguish four subspecies within *G. luteum*. In the list of synonyms given above, the terms *argentatum*, *flavidum* and *montanum* denote both subspecies and varieties, but more recent taxonomic approaches prefer subspecies to variety (Rutkowski, 2011; Dvořáková, 2000; Mirek et al., 2002; Govaerts et al., 2010). Some authors have even proposed to raise those taxa to species rank (Dvořáková, 2000; Rosenbaumová et al., 2004; Castroviejo et al., 2010).

Individual subspecies of *G. luteum* (or species within the genus *Galeobdolon*) are identified on the basis of morphological features, both quantitative and qualitative, which have been listed in detail by Dvořáková (2000) and Rosenbaumová et al. (2004). To illustrate, bract characteristics and maximum number of flowers were selected as quantitative features with the highest discriminant power to distinguish subsp. *galeobdolon* from subsp. *montanum* (Rosenbaumová et al., 2004). In the case of subsp. *argentatum*, a key distinguishing feature is the year-long presence of leaves and bracts (excluding the uppermost) with a distinct silvery pattern (two bands along the midrib) (Dvořáková, 2000). Other significant features include the size of flowers and nutlets as well as bract shape (Dvořáková, 2000; Rosenbaumová et al., 2004). Recent SEM observations of the nutlet surface gave new diagnostic features. That study demonstrated that epidermal cell shape and cell wall ornamentation are distinctive features of the examined taxa. The most distinctive nutlets turned out to be those of *G. luteum* subsp. *montanum*. Nutlets of subsp. *argentatum* and subsp. *luteum* were also easy to distinguish on the basis of sculpture (Czarna and Bednorz, 2011). Chromosome number is yet another diagnostic character for the species. The chromosome number for subsp. *galeobdolon* and *flavidum* is $2n=18$, and for subsp. *argentatum* and *montanum* $2n=36$. A double number of chromosomes in the latter two subspecies points to their hybrid or allotetraploid origin (Bendiksby et al., 2011b). However, clear arguments to support

separation as species have not been presented to date.

The names *Galeobdolon endtmanii* or, depending on the taxonomic approach, *L. galeobdolon* subsp. *endtmanii* or *Lamium endtmanii*, are also found in literature. Karyological and morphometric research suggests that the plants attributed to this taxon do not merit any separate taxonomic rank and should be merged with *G. luteum* subsp. *montanum* (Rosenbaumová et al., 2004). This suggestion is also supported by ultrastructural analyses of nutlet surfaces that did not reveal any diagnostic features distinguishing *G. endtmanii* from *G. luteum* subspecies (Czarna and Bednorz, 2011).

MATERIALS AND METHODS

PLANT MATERIAL AND DNA EXTRACTION

Our study included 15 taxa: four subspecies of *Galeobdolon luteum*, 10 taxa representing the genus *Lamium*, and *Glechoma hederacea* as outgroup. Altogether 29 individuals were examined, some of them collected during field research and some taken from herbarium collections (Appendix 1). Total genomic DNA was extracted from plant material. Leaves were grated in a Mini-Beadbeater-1 tissue disruptor and then treated with the Genomic Mini AX Plant SPIN kit (A&A Biotechnology) following the manufacturer's protocols.

AMPLIFICATION AND SEQUENCING

Chloroplast genome analysis provides valuable data for phylogenetic reconstruction in plants (Baldwin, 1992), but as demonstrated by a number of authors, using only cpDNA sequences to verify the relationships among species may result in significant errors due to introgression or hybridization events (Rieseberg and Soltis, 1991; Doyle, 1992). The results from cpDNA and nuclear DNA analyses should be compared to eliminate this problem (Baldwin, 1992). We analyzed the sequential variation of four different DNA markers representing two genomes. Three of them represent the plastid genome (*accD*, *rpoC1*, *trnH-psbA*) and the internal transcribed spacer (ITS) is a nuclear DNA marker. Two of the analyzed sequences are coding sequences: *accD* encodes the beta-carboxyl transferase subunit of acetyl-CoA and *rpoC1* encodes the β subunit of chloroplast DNA-dependent RNA polymerase (PEP). The third plastid region, *trnH-psbA*, is a noncoding intergenic spacer. The internal transcribed spacer refers to two regions of the nuclear genome separating 18S, 5.8S, and 26S rRNA genes. Although ITS does not code any protein, research showed that it plays an important role in cleaving

TABLE 4. Primers used for amplification and sequencing of the analyzed DNA regions

Region	Forward primer's sequence (5'- 3')	Reverse primer's sequence (5'- 3')
<i>accD</i>	AGTATGGGATCCGTAGTAGG	TTTAAAGGATTACGTGGTAC
ITS	ACGAATTCATGGTCCGGTGAAGTGTTCG	TAGAATTCCCCGGTTCGCTCGCCGTTAC
<i>rpoC1</i>	TATGAAACCAGAATGGATGG	GAAAACATAAGTARRCGWGC
<i>trnH-psbA</i>	GTTATGCATGAACGTAATGCTC	CGCGCATGGTGGATTACAAATC

the rRNA genes from the transcript of the whole ITS/rRNA region (Baldwin, 1992). All these regions are candidate DNA barcodes for plants. They are a valuable tool in phylogenetic studies on bryophytes and vascular plants.

The DNA fragments were amplified in a 20 μ l volume containing 20 mM $(\text{NH}_4)_2\text{SO}_4$, 50 mM Tris-HCl (pH 9.0 at 25°C), 1.5 mM MgCl_2 , 1 μ l BSA, 200 μ M of each dNTP, 1.0 μ M of each primer, one unit of TFL polymerase (Epicentre) and 10–20 ng DNA template. The reactions were performed under the following conditions: 5 min initial denaturation at 94°C; 45 s denaturation at 94°C (40 times); annealing – 50 s at 52°C for *trnH-psbA*, 55°C for *rpoC1*, 56°C for *accD*, 58°C for ITS (40 times); 1.5 min elongation at 72°C (40 times); 7 min final elongation at 72°C. Finally the amplification products were visualized on 2% agarose gel with GelView (InvitrogenTM) staining. Purified PCR products were sequenced in both directions using an ABI BigDye 3.1 Terminator Cycle Kit with the same primers and then visualized using an ABI Prism 3130 Automated DNA Sequencer (Applied Biosystems[®]).

For amplification and sequencing of *accD* and *rpoC1* we used the primers from the Royal Botanical Garden on the Kew website, for amplification and sequencing of *trnH-psbA* the primers of Sang et al. (1997), and for ITS the primers of Sun et al. (1994). The primer sequences are given in Table 4.

DATA ANALYSIS

Electrophoretograms were edited and assembled using Sequencher 4.1.4 (Gene Codes Corporation). The assembled sequences were aligned and manually adjusted using BioEdit 7 (Hall, 1999). Regions of ambiguous alignment and incomplete data at the beginning and end of sequences were excluded from the analyses. Minimum evolution (ME) and maximum parsimony (MP) analyses were done using MEGA v.5 (Tamura et al., 2011). In the ME method a maximum composite likelihood model was used and the initial tree was obtained by neighbor-joining. The tree inference was made with the close neighbor interchange (CNI) algorithm (Nei and Kumar,

2000) at the search level of 2. In MP the CNI was also applied at the level of 2 and the number of initial trees was 10. The phylogeny was tested both in ME and MP with the bootstrap method (Felsenstein, 1985) with 1000 iterations.

Bayesian inference phylogenetic analyses was done using MrBayes v. 3.1.2 (Hulsenbeck and Ronquist, 2001) with the priors set according to the output of jModelTest 0.1.1 (Posada, 2008). Optimal models of nucleotide substitution for chloroplast and nuclear sequences were selected on the basis of Bayesian information criterion (BIC) results. The parameters of the likelihood model applied for the nuclear region were adequate for a general time reversible model with a gamma-shaped distribution of rates across sites (GTR+ Γ), (nst=6). For chloroplast sequences all substitutions had the same rate across sites (nst=1) corresponding to the Felsenstein (1981) model (F81). The MCMC algorithm was run for 1,250,000 generations for ITS and 1,500,000 generations for chloroplast sequences. There were six incrementally heated chains sampling one out of every 100 generations of random trees. The standard deviation of split frequencies (SDSF) was monitored to test when the Markov Chain converged. The generations prior to the point at which SDSF stabilized at a level below 0.01 were discarded as burn-in. The remaining generations were used to construct the Bayesian consensus tree.

Incongruence between the ITS and cpDNA data was assessed by comparing clade support on the consensus trees. For example, if species A was included in clade A with significant bootstrap support based on interference in the ITS region, but resolved as a member of clade B with significant support based on the cpDNA sequences, the phylogenetic trees based on these loci were considered incongruent. To identify incongruence in phylogenetic signals we used the 70% bootstrap criterion. Agreement between the trees obtained by different phylogenetic methods was analyzed in the same way. As another measure of distinctness, the number of fixed nucleotide differences among the representatives of *Galeobdolon luteum* and the species belonging to *Lamium* was estimated using the Sites program (Hey and Wakeley, 1997).

RESULTS

ITS REGION

The analyzed sequence described as ITS consists of ITS1, ITS2, a sequence for 5.8S rRNA lying between these two spacers, a small fragment of 18S rRNA (27 bp) and 28S rRNA (25 bp). The alignment had a total length of 704 bp. The shortest amplified sequence (662 bp) was found in *G. luteum* and *G. hederacea*, and the longest (687 bp) in *L. incisum*. All indels were found within the spacers. There were 97 parsimony-informative sites within the alignment, only 5 of them within coding regions. The MP method revealed the 150 most parsimonious trees, with a tree length of 156, a consistency index (CI) of 0.792683 and a retention index (RI) of 0.933852.

All three methods generated similar trees with *G. luteum* forming a well-supported clade (Fig. 1). Bayesian inference was used to establish the clade credibility value at 1.00, with 98% MP and 92% ME. Clade distinctness was also asserted by the number of fixed nucleotide differences: 20 nucleotide differences, including 15 substitutions and 5 indels, were found between five representatives of *G. luteum* and the other *Lamium* species. Five substitutions and 2 indels were observed in the ITS1 region, and 8 substitutions and 3 indels in the ITS2 region. Three substitutions were observed within the genes coding for 5.8S rRNA. The detected indels had the length of 1–3 nucleotides. The remaining species of the genus *Lamium* formed two more fairly well-supported clades (Fig. 1). These results mean that an ITS-based tree does not distinguish two sister groups as would be expected in a comparison of two genera.

Two well-supported clades are identified in the *G. luteum* group: *G. luteum* subsp. *flavidum* with *G. luteum* subsp. *montanum*, and the nominative subspecies with *G. luteum* subsp. *argentatum* (Fig. 1). It should be noted that subsp. *argentatum* and *montanum* have a double number of chromosomes ($2n=36$), indicating that each clade contains one subspecies with the basic chromosome set and one subspecies with a double number of chromosomes. The division of the subspecies into two clades was confirmed by a high number of fixed substitutions (no indels were found). On average there were 9.25 pairwise differences between the taxa from the two clades, and much fewer within-clade pairwise differences. *G. luteum* subsp. *galeobdolon* and *G. luteum* subsp. *argentatum* differed by only 2 substitutions, and *G. luteum* subsp. *flavidum* differed from *G. luteum* subsp. *montanum* by 3.

CHLOROPLAST DNA

The amplified fragment of *accD* had a total length of 324 bp and revealed 23 variable sites. The align-

ment was also 324 bp long, as there were no indels in the analyzed gene fragment. The amplified partial sequence of *rpoC1* had a total length of 701 bp. There were no differences in the length of the sequence between the analyzed species. The length of the *trnH-psbA* spacer ranged from 232 bp in *L. album* to 279 bp in *G. luteum* and 341 bp in *G. hederacea*. The alignment had a total length of 351 bp. Within the three analyzed chloroplast regions there were 57 parsimony-informative sites altogether: 5 for *accD*, 15 for *rpoC1* and 37 for the *trnH-psbA* spacer. The maximum parsimony method applied to all the three chloroplast regions resulted in 1117 most parsimonious trees of 141 steps, with CI=0.885714 and RI=0.963470.

Analyses of chloroplast regions carried out by the MP method and Bayesian inference produced trees of similar topology. In both trees, representatives of the *G. luteum* species were grouped in a fairly well-supported clade (0.74 credibility value and 58% bootstrap support), but the analyzed clade was not distinguished from the *Lamium* species. The tree developed based on cpDNA data points to a close relationship between *G. luteum* and *Lamium*. A close relationship between *G. luteum* and *L. album* was determined by Bayesian inference (credibility value 0.81) (Fig. 2). The results delivered by the ME method did not contribute to the analysis of phylogenetic relationships within the species because clade support was too weak and the consensus tree was full of polytomies. A strong phylogenetic signal is visible only in a few instances (Fig. 2).

The analysis of the polymorphic sites of *G. luteum* and the remaining *Lamium* species did not include *L. album* because high genetic similarity between species sharing the same clade could understate the result. Despite exclusion of *L. album*, only three fixed nucleotide differences were found. The differences were substitutions within the *trnH-psbA* spacer. In the cpDNA phylogram, the division of *G. luteum* subspecies into two groups was less clear (Fig. 1). Only the clade comprising subsp. *galeobdolon* and *argentatum* was well supported (credibility value 0.96).

DISCUSSION

In verifying the taxonomic status of species and improving their classification, the results from other diagnostic methods should be taken into account. This has been done in, for example, five East Asian species of uncertain taxonomic status: *L. chinense* Benth. [syn.: *Galeobdolon chinense* (Benth.) C.Y.Wu], *L. kwangtungense* C.Y.Wu (syn. *G. kwangtungense* C.Y.Wu), *L. szechuanense* C.Y.Wu (syn. *G. szechuanense* C.Y.Wu), *L. yangsoense* Y.Z.Sun (syn. *G. yangsoense* Y.Z.Sun) and

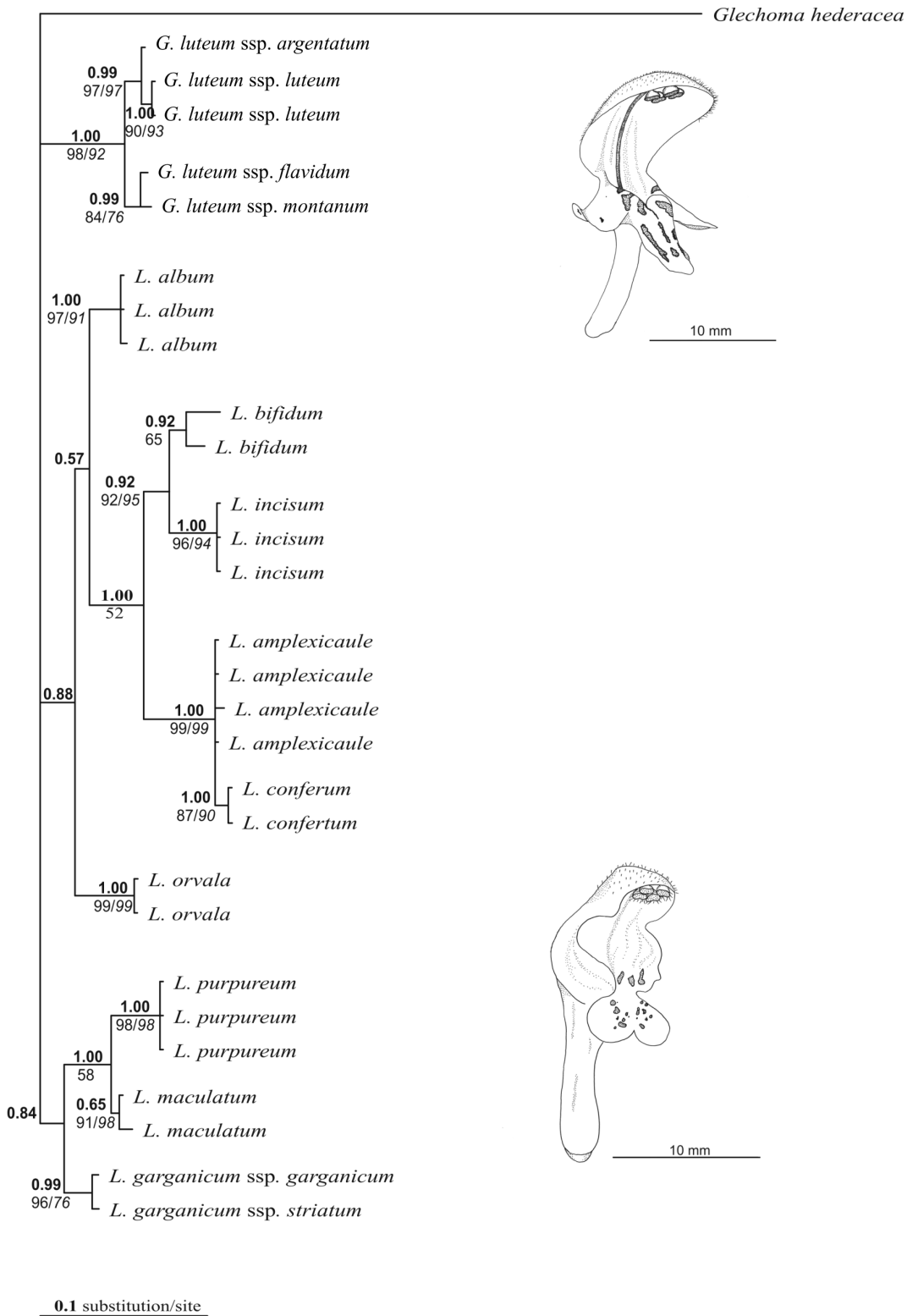


Fig. 1. 50% majority-rule consensus phylogram from Bayesian analysis of ITS sequence data. Credibility values above 0.50 are given in boldface. Bootstrap values of clades supported by maximum parsimony and minimal evolution analysis are given below branches (MP/ME, in italics). Upper image shows crown of *G. luteum* with characteristic tripartite lower lobe. Lower image shows crown of *L. purpureum*, the type of the genus *Lamium*.

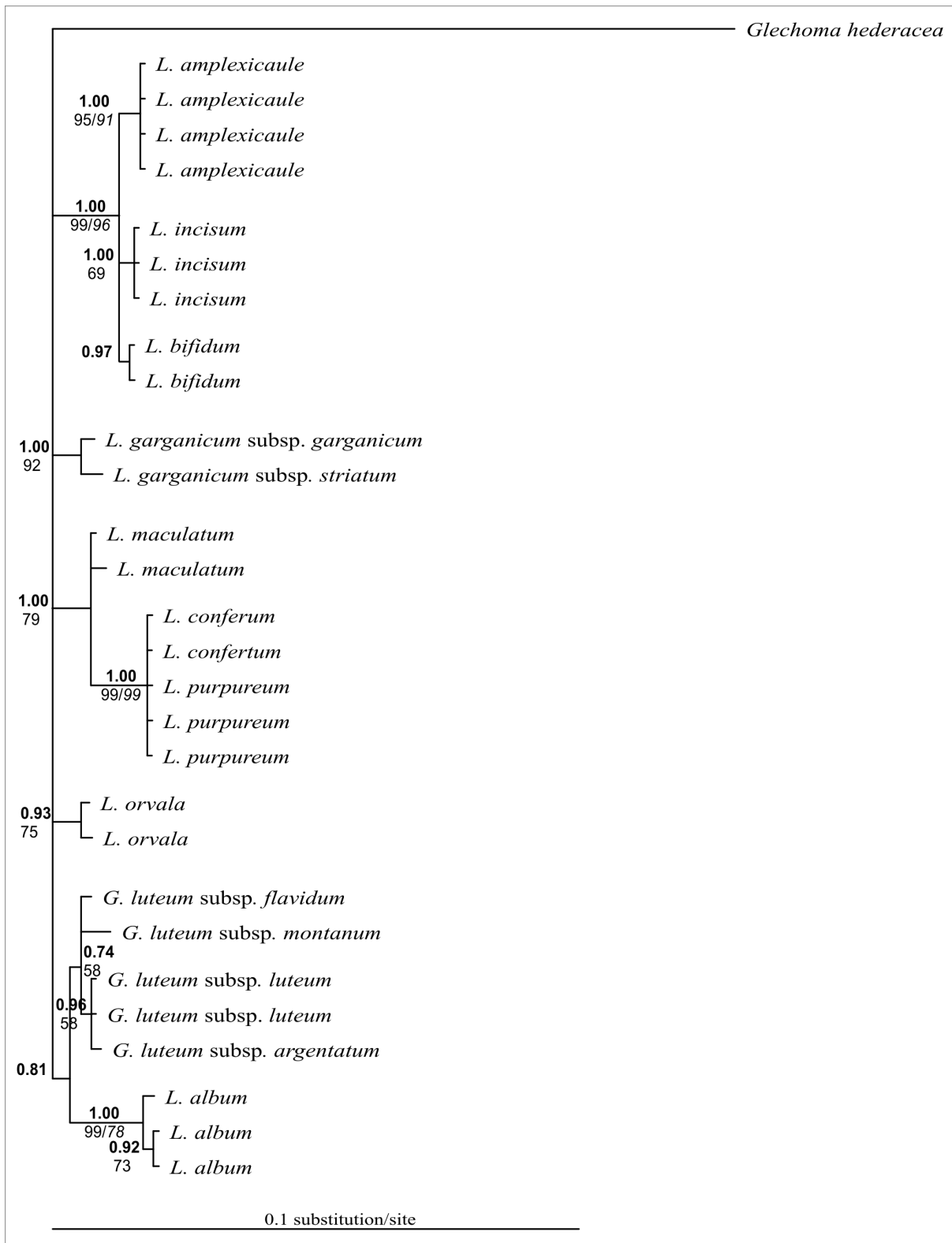


Fig. 2. 50% majority-rule consensus phylogram from Bayesian analysis of chloroplast sequence data (*accD*, *rpoC1*, *trnH-psbA*). Credibility values above 0.50 are given in bold. Bootstrap values of clades supported by maximum parsimony and minimal evolution analysis are given below branches (MP/ME, in italics).

G. tuberiferum (Makino) C.Y. Wu (Govaerts et al., 2010). Based on an analysis of morphological features, Ryding (in Harley et al., 2004) suggested that those East Asian species are more likely to belong to the genus *Matsumurella* rather than *Lamium* or *Galeobdolon*. According to Ryding, their distinctive features are prominent and rounded lateral corolla lobes vs. triangular acute or short acute lobes in *Lamium* (and *Galeobdolon*). Ryding's findings indicate that the analyzed groups are distinguished by a single morphological feature, indicating significant morphological similarity. Ryding's assertion was supported by Bendiksby et al. (2011a), who analyzed the molecular tree based on chloroplast sequences (*matK*, *rps16*, *trnL*, *trnL-F*) and concluded that the 'Matsumurella group' is extraneous to *Lamium* and *Lamiastrum*. The presented arguments were strong enough to justify transferring those species to the genus *Matsumurella*.

Identification of *G. luteum* is based on three main morphological features distinguishing it from *Lamium* species: yellow color of the corolla in *Galeobdolon* vs. purple, pink or white in *Lamium*; the presence of three lobes of roughly equal size in the corolla's lower lobe (*Galeobdolon*) vs. one well-developed lobe with or without small lateral lobes (*Lamium*); and triangular or oblong shape of lobes (*Galeobdolon*) vs. obcordate or broadly obovate lower lip (*Lamium*) (Sychowa, 1967; Ball, 1972; Szafer et al., 1986; Rutkowski, 2011) (Fig. 1). It is debatable whether those key features are enough to place them in separate genera. Opinions are divided, and taxonomists take different approaches to the problem. However, it seems that the morphological differences between *Galeobdolon* and *Lamium* are even more extensive than those distinguishing *Lamium* from *Matsumurella*. In our molecular study this morphological distinctiveness between genera was not confirmed by their genetic distance.

Our analysis of cpDNA sequences (Fig. 2) did not reveal significant differences between *G. luteum* and the dead-nettle species, and confirmed the results from other studies investigating the chloroplast genome (Kaufmann and Wink, 1995; Sheen et al., 2010; Bendiksby et al., 2011b) which found close relationships between the genera *Lamium* and *Galeobdolon*. Sheen et al. (2010) proposed to group the two genera with *Wiedemannia* as part of the tribe Lamieae, and some of the same researchers made them part of the genus *Lamium* in a subsequent paper (Bendiksby et al., 2011a).

In an analysis of nuclear data (*NRPA2* gene and 5S-NTS), Bendiksby et al. (2011b) demonstrated that *G. luteum* is distinct. Their study indicates that *G. luteum* forms a sister group with a strongly supported clade comprising the remaining *Lamium* species, and that *Lamium* forms a monophyletic group regardless of whether *G. luteum* is included.

Such a clear distinction between the genera was not found in our analysis of the nuclear region because dead-nettle taxa do not form a monophyletic clade.

In view of the observed incongruence between genomes, we conclude that our findings support continued separation of Yellow Archangel from the *Lamium* species. However, the separation is not so well supported by our cpDNA analysis. The chloroplast genome has a lower rate of evolution than the nuclear genome (Wolfe et al., 1987), since the vast majority of cpDNA genomes have lower rates of nucleotide substitution and gene rearrangement (Avice, 1994). The divergence of phylogenetic lines is easier to observe in nuclear genomes.

Our genetic analyses indicate that *G. luteum* is not genetically distant enough from *Lamium* to qualify as a separate genus. This study seems to validate the approach of taxonomists who include Yellow Archangel in the genus *Lamium*. The proposal to raise the four *G. luteum* subspecies to the rank of separate species is not supported by molecular analysis. The division of those subspecies into two groups, based on analyses of different DNA regions (Bendiksby et al., 2011b), suggests that the studied polyploid species have the following origin: *G. luteum* subsp. *argentatum* evolved from *G. luteum* subsp. *montanum*, and *G. luteum* subsp. *flavidum* evolved from *G. luteum* subsp. *galeobdolon*. Further work is needed to verify this hypothesis.

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APPENDIX 1. Specimens used in the study and GenBank accession numbers for DNA sequence data. Accessions marked with collection year in italics were collected by Krawczyk during field research

Taxon	ID No.	Country	Year	Voucher number	<i>accD</i>	ITS	<i>rpoC1</i>	<i>trnH-psbA</i>
<i>Lamium album</i>	218	Poland	2010	-	JX073934	JX073963	JX073992	JX074021
<i>L. album</i>	220	Poland	2010	-	JX073935	JX073964	JX073993	JX074022
<i>L. album</i>	222	Poland	2010	-	JX073936	JX073965	JX073994	JX074023
<i>L. amplexicaule</i>	112	Poland	2009	-	JX073937	JX073974	JX073995	JX074024
<i>L. amplexicaule</i>	153	Poland	2010	-	JX073940	JX073977	JX073998	JX074027
<i>L. amplexicaule</i>	165	Poland	2009	-	JX073938	JX073975	JX073996	JX074025
<i>L. amplexicaule</i>	177	Poland	2009	-	JX073939	JX073976	JX073997	JX074026
<i>L. bifidum</i>	271	Spain	2005	(COA) 33936	JX073954	JX073967	JX074012	JX074041
<i>L. bifidum</i>	272	France	1990	(H) 1670212	JX073955	JX073966	JX074013	JX074042
<i>L. confertum</i>	169	Russia	1894	(UMK) -	JX073946	JX073984	JX074004	JX074033
<i>L. confertum</i>	256	Finland	2003	(TUR) 575205	JX073947	JX073985	JX074005	JX074034
<i>L. garganicum</i> subsp. <i>garganicum</i>	275	Italy	2002	(BR) 9549159	JX073951	JX073980	JX074009	JX074038
<i>L. garganicum</i> subsp. <i>striatum</i>	276	Greece	1986	(H) 159838	JX073952	JX073981	JX074010	JX074039
<i>L. incisum</i>	33	Poland	2009	-	JX073942	JX073969	JX074000	JX074029
<i>L. incisum</i>	160	Poland	2010	-	JX073941	JX073968	JX073999	JX074028
<i>L. incisum</i>	161	Poland	2009	-	JX073943	JX073970	JX074001	JX074030
<i>L. maculatum</i>	181	Poland	2001	(OLS) 17229-30	JX073944	JX073978	JX074002	JX074031
<i>L. maculatum</i>	182	Poland	1987	(OLS) 12447-49	JX073945	JX073979	JX074003	JX074032
<i>L. orvala</i>	281	Italy	1991	(H) 1690069	JX073956	JX073982	JX074014	JX074043
<i>L. orvala</i>	282	Italy	1977	(TUR) 242936	JX073957	JX073983	JX074015	JX074044
<i>L. purpureum</i>	19	Poland	2009	-	JX073948	JX073971	JX074006	JX074035
<i>L. purpureum</i>	93	Poland	2009	-	JX073949	JX073972	JX074007	JX074036
<i>L. purpureum</i>	236	Poland	2010	-	JX073950	JX073973	JX074008	JX074037
<i>Galeobdolon luteum</i> subsp. <i>argentatum</i>	240	Portugal	1998	(H) 1713345	JX073931	JX073960	JX073989	JX074018
<i>G. luteum</i> subsp. <i>flavidum</i>	239	Austria	1996	(H) 1714140	JX073932	JX073961	JX073990	JX074019
<i>G. luteum</i> subsp. <i>luteum</i>	199	Poland	1998	(OLS) 14624	JX073929	JX073958	JX073987	JX074016
<i>G. luteum</i> subsp. <i>luteum</i>	216	Poland	2009	-	JX073930	JX073959	JX073988	JX074017
<i>G. luteum</i> subsp. <i>montanum</i>	238	Belgium	1988	(H) 1656894	JX073933	JX073962	JX073991	JX074020
<i>Glechoma hederacea</i>	215	Poland	2010	-	JX073953	JX073986	JX074011	JX074040