

Genome fingerprinting confirms the species status of the Loosestrifes *Lysimachia punctata* and *L. verticillata* (Primulaceae)

Yann Triponez¹, Begüm Şepitci² & Nadir Alvarez^{3,*}

¹Laboratory of Evolutionary Entomology, Institute of Biology, University of Neuchâtel, Rue Emile-Argand 11, 2000 Neuchâtel, Switzerland

²Hacettepe University, Department of Biology, 06800 Beytepe, Ankara, Turkey

³Department of Ecology and Evolution, University of Lausanne, Biophore, UNIL-Sorge, 1015 Lausanne, Switzerland

*Author for correspondence: nadir.alvarez@unil.ch

Background and aims – Despite their vicariant distribution and slightly distinct morphologies, the Dotted Loosetrife (*L. punctata* L.) and the Whorled Loosetrife (*L. verticillata* (Green) Hand.-Mazz.) have sometimes been treated as synonyms, or even confounded by several authors. Here we examine the genetic divergence of these two taxa in order to clarify their species status.

Methods – We apply AFLP genome fingerprinting to samples from eleven wild and two naturalized populations, and compile occurrence data from herbaria and public data depositories.

Key results – The distribution of genotypes in a multivariate space confirms the distinct species status of the two species, and highlights the absence of natural hybrids between the two in their native area. This result contrasts with the hybrid nature of naturalized populations from northern Europe, likely produced through horticultural practices.

Key words – AFLP, hybridization, *Lysimachia punctata*, *Lysimachia verticillata*, species status, vicariance.

INTRODUCTION

With 191 species described, the genus *Lysimachia* L. (Primulaceae) is one of the most speciose within Myrsinoideae (Hao et al. 2004). Whereas its paraphyletic status is suggested (Anderberg et al. 2007), several well-supported clades may serve as a basis for an assignment of species to new generic entities. This is for instance the case of a clade composed of the Moneywort *L. nummularia* L. and the Dotted Loosetrife *L. punctata* L. (referred to as *group B* by Anderberg et al. 2007), which shows unresolved relationships to the other *Lysimachia* clades.

Although not included in the phylogenetic analysis by Anderberg et al. (2007), the Whorled Loosetrife *L. verticillata* (Green) Hand.-Mazz. is expected to stand within the same group as *L. punctata* (Huxley et al. 1992), considering its strong similarity. Many ecological and morphological details provided by Leblebici (1978) and McAllister (1999) support this hypothesis: in the wild, the two species occupy similar wet habitats and only differ on subtle morphological traits such as petiole length, colour of petal base and inflorescence characters. McAllister (1999) however pointed out differences in sexual characters of naturalized populations in the British Isles: *L. punctata* is self-incompatible, in contrast to *L. verticillata*. Leblebici (1978) further emphasized their

non-overlapping native ranges: the Dotted Loosetrife occurs in eastern Europe and western Turkey, while the Whorled Loosetrife is found in northern and eastern Turkey, Caucasus, the Crimea and Northern Iran.

As emphasised by McAllister (1999), there was clearly confusion in the literature about the Dotted and the Whorled Loosestrifes. The Plant List (2013) currently accepts the names *Lysimachia punctata* L. and *L. verticillata* (Greene) Hand.-Mazz. for these two morphologically similar taxa. Among mentions found for the Whorled Loosetrife, only Huxley et al. (1992) used *L. verticillata* (sic), as Leblebici (1978) or McAllister (1999) used *L. verticillaris* Spreng. Interestingly, Ferguson (1972) earlier regarded *L. verticillaris* Spreng. as synonymous with *L. punctata*, as does the Plant List nowadays.

Lysimachia punctata, *L. verticillata* and *L. vulgaris* L., another species with a large Eurasiatic distribution, are widely cultivated and known as being naturalized in most of central and northern Europe (for the two first) and in North America (all of them). While cultivars of *L. vulgaris* are commonly named Garden Loosetrife, cultivars of *L. punctata* and *L. verticillata* are indifferently grouped under the generic Yellow Loosetrife or Garden Yellow Loosetrife, adding to the species confusion described above.

This study was motivated because similarities observed between the Dotted Loosestrife and the Whorled Loosestrife have not been genetically investigated so far. Here, we examine whether the two Loosestrifes *L. punctata* and *L. verticillata* are genetically differentiated, using AFLP genome fingerprinting. We compare their genotypes in their native areas as well as in naturalized populations from Northern Europe.

MATERIAL AND METHODS

In order to examine the genetic status of *L. punctata* and *L. verticillata*, we collected individuals across their native areas in mountainous regions surrounding the Black Sea, as well as from naturalized sites in northern Europe (fig. 1; see electronic appendix 1 for abbreviations). Eleven wild populations (with population codes in parentheses; three to five individuals per population) were sampled in Bulgaria (VIT, BEK), Greece (KON, KRA), Turkey (ERF, ILG, YUC), Georgia (BAK, TKI) and the Crimea (KRS, PER). Samples from two naturalized populations were collected in Norway (NOR) and Scotland (BAR). Our sampling was guided by the occurrence data of both species obtained from Turkish herbaria and the Turkish Plants Data Service (TÜBİVES), as well as from GBIF (see electronic appendix 1 and fig. 1). In order to scale the extent of genetic divergence between the two taxa, we included in the analysis *L. vulgaris* samples from 25 populations from Turkey to Poland (where *L. vulgaris* is sympatric both with *L. punctata* and *L. verticillata*,

see fig. 1) as an outgroup, this species clustering in another clade in all phylogenies published so far (Hao et al. 2004, Manns & Anderberg 2005, Anderberg et al. 2007).

One leaf per plant was cut and dried in silica-gel following Chase & Hills (1991). DNA was extracted with the DNeasy Plant Kit (Qiagen, Hilden, Germany). AFLP analysis was performed following Triponez et al. (2015), with the two primer combinations *EcoRI*-ACA/*MseI*-CTA and *EcoRI*-ATA/*MseI*-CAC. The final binary matrix was analyzed by principal coordinate analysis (PCoA) relying on the Jaccard similarity coefficient using the software Ginkgo 1.7.0 (De Caceres et al. 2007). Using GenAlEx 6.5 (Peakall & Smouse 2012), we estimated among species divergences and overall average heterozygosities per species using Nei's genetic diversity index, as well as further performed an Analysis of Molecular Variance (AMOVA) considering both the species and population (38 in total) levels.

RESULTS AND DISCUSSION

Raw genetic data are provided as an electronic complement in electronic appendix 2. AFLP analysis yielded 239 polymorphic loci (122 and 117 for primer pairs *EcoRI*-ACA/*MseI*-CTA and *EcoRI*-ATA/*MseI*-CAC, respectively), after removing those showing less than 95% of reproducibility. *Lysimachia punctata* (N = 24), *L. verticillata* (N = 28), and *L. vulgaris* (N = 115) show similar levels of diversity, with

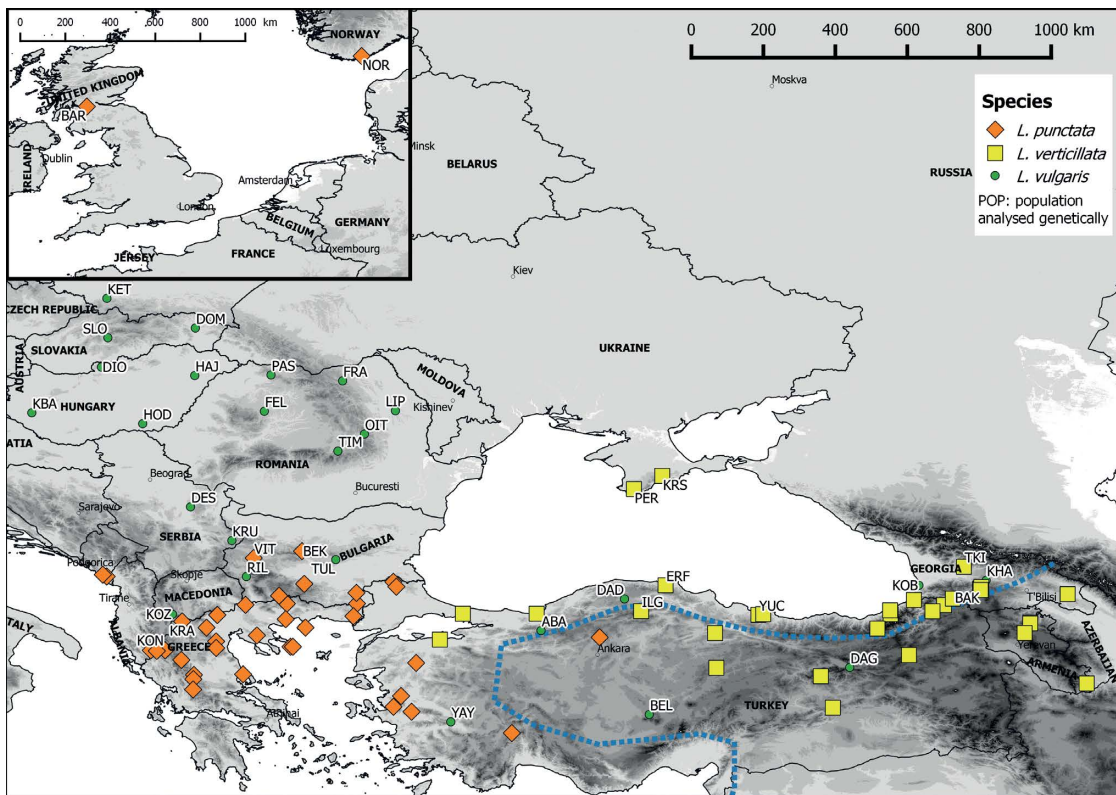


Figure 1 – Sampling, and occurrences from botanical databases, of *Lysimachia punctata* and *L. verticillata* across their native area (main map), also showing the location of the naturalized samples collected (smaller map). *L. vulgaris* populations sampled and used for scaling the extent of genetic divergence between taxa are also shown on maps. The limit between West Asiatic and East Mediterranean/Circumboreal floristic regions is represented by a blue-dotted line.

respectively 149, 146 and 147 polymorphic loci, and overall average heterozygosities of 0.184, 0.153 and 0.123, respectively. The extent of allele sharing among species is given as electronic appendix 3.

The genetic analysis confirms the species status of *L. punctata* and *L. verticillata*. The PCoA analysis of AFLP fingerprinting results (fig. 2) indeed reveals a strong genetic divergence among *L. punctata* individuals from southern Balkans (Greek and Bulgarian populations, in orange on fig. 1 and fig. 2), *L. verticillata* samples from northern, eastern and southern regions around the Black Sea (Crimean, Georgian and Turkish populations, in yellow in fig. 1 and fig. 2), as well as sympatric *L. vulgaris* individuals. Divergence among the three taxa is confirmed by Nei's genetic distances (0.09 between *L. verticillata* and *L. punctata*, 0.181 between *L. punctata* and *L. vulgaris* and 0.215 between *L. verticillata* and *L. vulgaris*). Because expected Nei's genetic distance between closely related plant species can be as low as 0.09 (Nosrati et al. 2015), we argue that the divergence observed between *L. punctata* and *L. verticillata* is compatible with the interspecific level, although – because a Nei's genetic distances of 0.09 is at the lower bound of the distance values expected for the inter-specific level – we cannot rule out that *L. verticillata* and *L. punctata* could produce viable hybrids in the area where both species are sympatric, i.e., in western Turkey (given that our genetic sampling did not cover this part of the species' distribution, further analyses should include samples from therein in order to clarify whether or not *L. verticillata* and *L. punctata* are able to hybridize). This

result is further confirmed by the AMOVA analysis, showing 56% of genetic variance explained among species, 12% among populations and 32% within populations. The relatively high variance at the population level could be due to hybrids found in several locations (see below, as well as fig.1).

From a biogeographic perspective, the split between *L. punctata* and *L. verticillata* may roughly match the boundary between the West Asiatic and East Mediterranean floristic regions (Manafzadeh et al. 2014), although *L. verticillata* is also marginally present northwards of the boundary along the southern coast of the Black Sea and in Crimea. Nonetheless, such a vicariant pattern between closely related species in the Anatolian Peninsula is also found in other biological groups, such as, for instance, the green lizards *Lacerta pamphylica* and *L. trilineata* (Ahmadzadeh et al. 2013), or the annelids *Spermophorodilus antiquus* and *S. vignai* (Omodeo & Rota 1999).

Our results thus confirm Leblebici's (1978) view of the species status of these two vicariant taxa, with *L. punctata* growing in south-eastern Europe and western Turkey, and *L. verticillata* (despite Leblebici wrongly names it *L. verticillaris*) being found in northern and eastern Anatolia, Caucasus or Crimea. Ranges of the two species do not clearly overlap as shown by our survey of Turkish herbarium specimens (see electronic appendix 1 and fig. 1). Still, a contact zone might exist in north-western Anatolia. Additional sampling of populations in appropriate habitats between Bursa and Ankara could detect such a contact zone. Finally, molec-

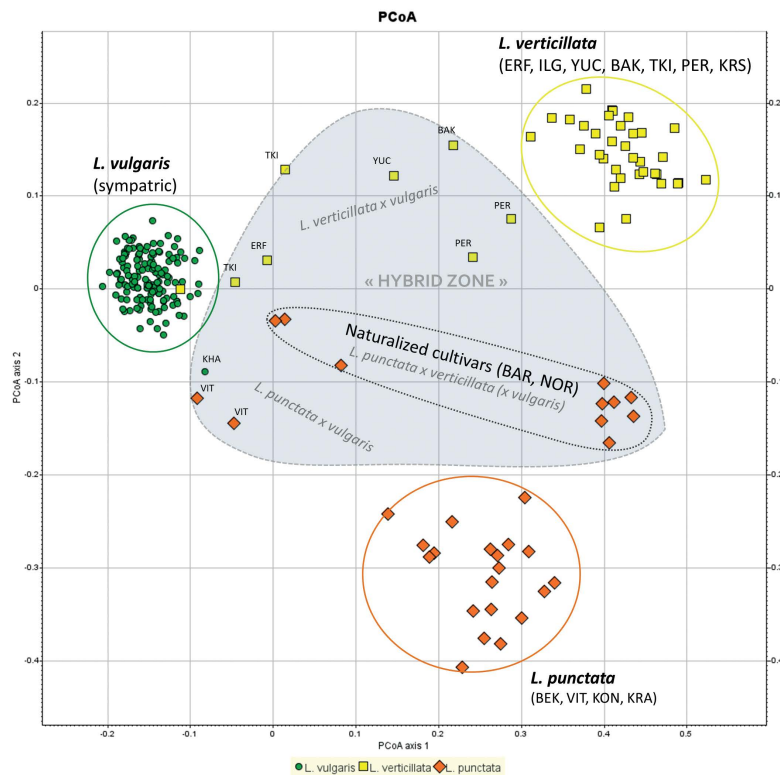


Figure 2 – Principal coordinate analysis (PCoA) plot of all analyzed samples, based on Jaccard similarity coefficient among genotypes. The two first axes explain 21% and 5% respectively of the total variance. Each point represents one individual, according to its morphological determination (green dots for *L. vulgaris*, yellow squares for *Lysimachia verticillata* and red diamonds for *L. punctata*).

ular dating based on DNA sequences rather than genotypes would be useful in order to define the spatio-temporal processes involved in the divergence between *L. punctata* and *L. verticillata*.

Last but not least, our study addresses interesting questions regarding hybridization (natural or artificial) between the three species. First, we detected no natural hybrids between *L. punctata* and *L. verticillata*. Only further genetic analysis of populations sampled in the contact zone could however exclude the chance of such a crossing to occur naturally. Second, some wild individuals intermediate between *L. punctata*, or *L. verticillata*, and the widely spread *L. vulgaris* may suggest possible natural hybridization. This could take place via cross-pollination mediated by shared oligolectic pollinators such as the oil-collecting bees *Macropis* (Hymenoptera: Melittidae) or by more generalist bee pollinators such as *Lasioglossum* (Hymenoptera: Halictidae), both observed pollinating these *Lysimachia* (Bassin et al. 2011, Triponez et al. 2015). Finally, we note that individuals of the Garden Loosestrife sampled in naturalized populations from Northern Europe (in UK and Norway, originally identified as *L. punctata*) are located halfway between the wild *L. punctata* and *L. verticillata* (see fig. 2: BAR, NOR). This typical hybrid position might suggest a recent history of artificial crossings between these two species, and even further introgression (natural or artificial) to such cultivars from *L. vulgaris*.

SUPPLEMENTARY DATA

Supplementary data are available at *Plant Ecology and Evolution*, Supplementary Data Site (<http://www.ingentaconnect.com/content/botbel/plecevo/supp-data>), and consist of the following: (1) localities analyzed in this study, with population code (for samples analyzed genetically), geographical coordinates, country and source of information for each observation (pdf); (2) raw genetic data of AFLP fingerprinting results (Excel spreadsheet); and (3) number of loci shared among species (over 239 polymorphic loci) (pdf).

ACKNOWLEDGMENTS

The authors are grateful to A. Espindola, I. Hiltbold, V. Joinel Alvarez, N. Joinel and D. Kharazishvili for their help during field collections, and A. Sarr for support with AFLP analysis. This research was supported by the Swiss National Science Foundation (grant no. 3100A0-116778/1).

REFERENCES

Ahmadzadeh F., Flecks M., Rödder D., Böhme W., Ilgaz Ç., Harris D.J., Engler J.O., Üzümlü N., Carretero M.A. (2013) Multiple dispersal out of Anatolia: biogeography and evolution of oriental green lizards. *Biological Journal of the Linnean Society* 110: 398–408. <http://dx.doi.org/10.1111/bij.12129>

Anderberg A.A., Manns U., Källersjö M. (2007) Phylogeny and floral evolution of the Lysimachieae (Ericales, Myrsinaceae): evidence from ndhF sequence data. *Willdenowia* 37: 407–421. <http://dx.doi.org/10.3372/wi.37.37202>

Bassin L., Alvarez N., Pellissier L., Triponez Y. (2011) Ecological niche overlap in sister species: how do oil-collecting bees *Macropis europaea* and *Macropis fulvipes* (Hymenoptera: Melittidae) avoid hybridization and competition? *Apidologie* 42: 579–595. <http://dx.doi.org/10.1007/s13592-011-0067-z>

Chase M.W., Hills H.H. (1991) Silica-gel: an ideal material for field preservation of leaf samples for DNA studies. *Taxon* 40: 215–220. <http://dx.doi.org/10.2307/1222975>

De Caceres M., Oliva F., Font X., Vives S. (2007) Ginkgo, a program for non-standard multivariate fuzzy analysis. *Advances in Fuzzy Sets and Systems* 2: 41–56.

Ferguson L.F. (1972) *Lysimachia* L. In: Tutin T.G., Heywood V.H., Burges N.A., Moore D.M., Valentine D.H., Walters S.M., Webb D.A. (eds) *Flora Europaea* 3: 26–21. Cambridge, Cambridge University Press.

Hao G., Yuan Y.-M., Hu C.M., Ge J.-X., Zhao N.-X. (2004) Molecular phylogeny of *Lysimachia* (Myrsinaceae) based on chloroplast trnL-F and nuclear ribosomal ITS sequences. *Molecular Phylogenetics and Evolution* 31: 323–339. [http://dx.doi.org/10.1016/S1055-7903\(03\)00286-0](http://dx.doi.org/10.1016/S1055-7903(03)00286-0)

Huxley A., Griffith M., Levy M. (1992) *The new Royal Horticultural Society Dictionary of gardening*. London, Royal Horticultural Society.

Leblebici E. (1978) *Lysimachia* L. In: Davis P.H. (ed.) *Flora of Turkey* 6: 135–138. Edinburgh, Edinburgh University Press.

McAllister H.A. (1999) *Lysimachia punctata* L. and *L. verticillaris* Sprengel (Primulaceae) naturalised in the British Isles. *Watsonia* 22: 279–281.

Manafzadeh S., Salvo G., Conti E. (2014) A tale of migrations from east to west: the Irano-Turanian floristic region as a source of Mediterranean xerophytes. *Journal of Biogeography* 41: 366–379. <http://dx.doi.org/10.1111/jbi.12185>

Manns U., Anderberg A.A. (2005) Molecular phylogeny of the Anagallis (Myrsinaceae) based on ITS, trnL-F and ndhF sequence data. *International Journal of Plant Sciences* 166: 1019–1028. <http://dx.doi.org/10.1086/449318>

Nosrati H., Feizi M.H., Razban-Haghighi A., Seyed-Tarrah S. (2015) Impact of life history on genetic variation in *Trifolium* (Fabaceae) estimated by ISSR. *Environmental and Experimental Biology* 13: 83–88.

Omodeo P., Rota E. (1999) Biogeographical patterns of terricolous oligochaetes in Turkey (Annelida: Clitellata: Lumbricidae, Enchytraeidae). *Biogeographia* 20: 61–79.

Peakall R.O.D., Smouse P.E. (2006) GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes* 6: 288–295. <http://dx.doi.org/10.1111/j.1471-8286.2005.01155.x>

The Plant List (2013) Version 1.1. Available from <http://www.theplantlist.org/> [accessed 1 Jan. 2016].

Triponez Y., Arrigo N., Espindola A., Alvarez N. (2015) Decoupled post-glacial history in mutualistic plant-insect interactions: insights for the yellow loosestrife (*Lysimachia vulgaris*) and its associated oil-collecting bees (*Macropis europaea* and *M. fulvipes*). *Journal of Biogeography* 42: 630–640. <http://dx.doi.org/10.1111/jbi.12456>

Manuscript received 15 Apr. 2015; accepted in revised version 5 Jul. 2016.

Communicating Editors: Olivier Raspé, Alexandra Ley, Elmar Robbrecht.