DNA barcoding of native Caucasus herbal plants: potentials and limitations in complex groups and implications for phylogeographic patterns

Parvin Aghayeva[‡], Salvatore Cozzolino[§], Donata Cafasso[§], Valida Ali-zade[‡],
Silvia Fineschi[|], Dilzara Aghayeva[‡]

‡ Institute of Botany, Azerbaijan National Academy of Sciences, Baku, Azerbaijan
§ Department of Biology, University of Naples Federico II, Complesso Universitario di Monte S. Angelo, Napoli, Italy
| CNR - Istituto di Scienze del Patrimonio Culturale, Sesto Fiorentino, Italy

Corresponding author: Dilzara Aghayeva (a_dilzara@yahoo.com)

Abstract

DNA barcoding has rapidly become a useful complementary tool in floristic investigations particularly for identifying specimens that lack diagnostic characters. Here, we assess the capability of three DNA barcode markers (chloroplast *rpoB*, *accD* and nuclear ITS) for correct species assignment in a floristic survey on the Caucasus. We focused on two herbal groups with potential for ornamental applications, namely orchids and asterids. On these two plant groups, we tested whether our selection of barcode markers allows identification of the "barcoding gap" in sequence identity and to distinguish between monophyletic species when employing distance-based methods. All markers successfully amplified most specimens, but we found that the rate of species-level resolution amongst selected markers largely varied in the two plant groups. Overall, for both lineages, plastid markers had a species-level assignment success rate lower than the nuclear ITS marker. The latter confirmed, in orchids, both the existence of a barcoding gap and that all accessions of the same species clustered together in monophyletic groups. Further, it also allowed the detection of a phylogeographic signal.The ITS marker resulted in its being the best performing barcode for asterids; however, none of the three tested markers showed

high discriminatory ability. Even if ITS were revealed as the most promising plant barcode marker, we argue that the ability of this barcode for species assignment is strongly dependent on the evolutionary history of the investigated plant lineage.

Keywords

Introduction

DNA barcoding in botany has rapidly spread as a reliable tool for the accurate identification of plant species or genus (Hebert et al. 2004), as well as for determining the origin of plants and their derivatives (Galimberti et al. 2019, Saravanan et al. 2019). Several studies highlighted the potential ecological applications of DNA barcoding in biodiversity assessments of both existing and past communities (Valentini et al. 2009). It was largely utilised in studies on local floras and plant communities for identifying specimens that are hard to recognise by morphological characters or that lack diagnostic floral characters (such as rarely blooming species or species with a short blooming period and/or brief juvenile stages) including identification of cryptic species (Xu et al. 2018). DNA barcoding allows potentially higher levels of species discrimination, particularly at regional floristic level; in fact, a geographically-restricted context usually contains fewer closely-related species than a comprehensive taxonomic treatment (Kress et al. 2009). At a local scale, the approach is particularly reliable when combined with the development of localised barcoding libraries for determining the identity of unknown samples (Chase and Fay 2009, Kress et al. 2009). Accordingly, the capacity of DNA barcoding in resolving species in local floras has been tested in many plant groups, including species-rich tropical communities (Ebihara et al. 2010, Burgess et al. 2011, Costion et al. 2016). These studies have also demonstrated that combined chloroplast and nuclear markers provided additional discriminatory power and increased percentage of success in species-level assignment, compared to the more traditional two-locus (*rbc*L and *mat*K) barcode (Vijayan and Tsou 2010). Due to the high rate of nucleotide substitution, the relatively easy amplification and the large sequence data already available, the internal transcribed spacer (ITS) regions of the nuclear ribosomal cistron (18S-5.8S-26S) have been very successful at species-level discrimination across flowering plants (Li et al. 2011, Feng et al. 2016, Hosseinzadeh-Colagar et al. 2016a). Nuclear barcodes are particularly useful for cases of recent hybridisation or ongoing introgression, because they can recover different allelic variants from a sample (Chase and Fay 2009). Thus, nuclear markers have been usually combined with (haploid) plastid markers in most DNA barcoding studies (Hosseinzadeh-Colagar et al. 2016b, Castro et al. 2015). Indeed, the adaptation of a multi-locus barcoding system, with at least two markers, each representing a distinct DNA source as nuclear and organellar genome, could contribute to the gathering of independent evidence of the species attribution and accessions relationships from independent gene trees (Moore 1995, Hu et al. 2015). Finally, barcode markers may also eventually show consistent intra-specific

variability (Hollingsworth et al. 2011). In that case and with a sampling representative of species distribution, haplotypic structure within a species can allow allocation of an individual to a geographic region and identify potential phylogeographic routes (Huemer and Hebert 2011).

The Caucasus represents one of the twenty-six biodiversity hotspot areas worldwide and has been the subject of botanical investigation since the beginning of the last century (Grossheim 1949, Karjagin 1928, Tutayuk et al. 1961). Recent research confirmed the Caucasus as part of the European flora (Bohn et al. 2007); indeed, many European plant lineages have close relatives in this region, including several domesticated plant species. The Caucasian flora represents a wonderful source of new food and medicinal plants and of new ornamentals with high adaptation potential in European gardens. Herbaceous monocots and dicots, particularly ornamental ones, are very numerous in the flora of the Caucasus and are characterised by low maintenance requirements. Indeed, these plants display high tolerance to environmental stresses as required for ornamental plants in the Mediterranean regions (Heywood 2003, Gray and Brady 2016).

Here, we employed DNA barcoding with the aim of investigating and quantifying plant diversity in the Quba and Qusar districts of Azerbaijan Caucasus. DNA-based methods are being increasingly used in floristic analyses, because they are not limited by taxonomic hindrances, such as: missing morphological features at any life stage (Wells and Stevens 2008, Ebihara et al. 2010); absence of distinctive identification characters in young or immature plants; and homoplasy of some characters (Vences et al. 2005). Nevertheless, the approach has some limitations when applied in the same critical groups as herbal species, particularly in the ability of species discrimination when closely-related species are examined (Chase and Fay 2009, Hubert and Hanner 2015). Here, we focused on two lineages, orchids and asterids, which are particularly interesting as they contain many ornamental species. We tested the potential of DNA barcodes for identifying unknown plant specimens and for identifying phylogenetic/phylogeographic relatedness with allied species and populations of other geographic origins. For this aim, we chose a combination of nuclear and plastid barcodes (ITS and chloroplast *rpoB*, *accD*), because DNA barcoding is particularly challenging when hybridisation might occur in conjunction with potential plastid capture or when lineage sorting has not yet been completed because of recent, rapid radiation (Fazekas et al. 2009, Chen et al. 2010), as expected in orchids and asterids. In particular, we tested whether the selection of barcode markers allows: i) the identification of the "barcoding gap" (Meyer and Paulay 2005) i.e. that the variation of the nucleotide sequences within species is lower than the differences amongst species and ii) the distinction between species, based on monophyletic clustering in distance-based neighbour-joining (NJ) trees (Hebert et al. 2004).

Material and methods

Study area. Qusar and Quba districts are located between 500–4466 m above sea level in the in the south macro-slope of the Greater Caucasus and north-eastern part of Azerbaijan. These districts spread along various altitudinal zonations (foothills, low, middle and high

mountain zones, subalpine, alpine habitats) and represent the richest floristic part of the country. The climate of the districts in summer is dry in the meadows and moderately hot in the foothills, whereas it is cold and very humid in the highlands and winter is usually cold. In the past couple of decades, increasing anthropogenic impacts, along with climate change, has contributed to the ecosystem degradation in these two districts.

Sampling. Approximately 500 ornamental herb specimens were collected during a floristic sampling campaign from 2012-2018 and were identified by means of morphological traits as belonging to 229 taxa, which are detailed as: 23 orders, 39 families and 129 genera. Morphologic identification was performed either by visual analysis or by using a dissection microscope, based on reliable diagnostic characters. Available checklists and recent literature on local floras (Karjagin 1928, Karjagin 1950, Aghayeva et al. 2018, Alizade et al. 2019, Tutayuk et al. 1961) were utilised as reference. The species status was further checked in the "World Flora Online" (http://www.worldfloraonline.org). Within this floristic survey, we selected altogether 54 accessions which were not clearly classified according to distinctive morphological characters. Thirty out of fifty-four accessions were roughly classified as Orchids and twenty-four accessions were roughly classified as Asterids. A small portion of leaf was preserved in silica gel and a barcode approach was performed as described below. All sampled specimens were collected in a herbarium within the Herbarium of the Institute of Botany, ANAS (BAK). We also had access to twenty herbarium vouchers of orchids and asterids, previously sampled from the same region and collected a small portion of dry specimens for performing the same barcode analysis.

DNA isolation, amplification and sequencing. Dried leaves from both field collection and herbarium samples were ground in a Tissue-lyser (Qiagen) and total DNA was extracted using GenElute™ Plant Genomic DNA Miniprep Kit (Sigma) following the manufacturer's instructions. The nuclear ribosomal DNA (internal transcribed spacer regions ITS1 and ITS2) was amplified with primers described by Aceto et al. 1999). For plastid barcode analysis, the two coding regions *rpoB* (RNA polymerase subunit) and *accD* (acetyl-CoA carboxylase subunit) were amplified with specific primers (sequences available at http://www.kew.org; barcoding/protocol.htlm). All PCRs were performed in a final reaction volume of 25 μl using about 10 ng of template DNA, 200 mM of each dNTP, 10 pmol of each of the two primers, 1× *Taq* buffer (50 mM KCl, 10 mM Tris–HCl pH 9.0), 1.5 mM MgCl$_2$ and 0.3 U of *Taq* polymerase (Sigma). Amplification of all barcodes was performed using the following protocol: initial denaturation at 94°C for 3 min, 35 cycles of denaturation at 94°C for 30 s, annealing at 53°C for 45 s and extension at 72°C for 1 min, followed by a final extension at 72°C for 7 min and final hold at 4°C. Amplification products were visualised on a 1.5% agarose gel and photographed after ethidium bromide staining. All successfully amplified DNA fragments were purified using the Clean Sweep PCR Purification Kit (Life Technology), following the manufacturer's instructions and then sequenced in both directions using a modification of the Sanger dideoxy method as implemented in a double-stranded DNA cycle sequencing system with fluorescent dyes. Sequence reactions were then run on a 3130 Automated sequence system (Applied Biosystem).

Sequence editing and alignment were performed by using BioEdit v.7.2.0 (Hall 2018). The species discrimination ability of each barcode marker was evaluated using GenBank (http://www.ncbi.nlm.nih.gov), a public available nucleotide sequences database. For species assessment, the database was screened for the presence of each of the marker sequences at the species or genus level relative to our dataset, using BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi). We considered as correct assignment when the query sequence has at least 99.5% of identical sites to the reference sequences in the database and when the top Bit-Score obtained in the GenBank matched the name of a single species. When the closest reference sequence scored lower than 99.5%, the result was considered as incomplete identification and imputable to the absence of the specific reference sequence in the database. Instead, when multiple reference sequences (i.e. from different species) shared the same top Bit-Score to the query sequence, the result was considered as incomplete identification due to insufficient discrimination power of the selected barcode.

Generated sequences and closest reference sequences (i.e. those identified by using BLAST and assigned to the same species) were aligned by using the MUSCLE programme in Mega X. For each barcode marker, a distance-based neighbour-joining (NJ) tree was then built with the Maximum Composite Likelihood model, uniform rates amongst sites and pairwise deletion in the gaps, for giving a graphic representation of the genetic distances within and amongst species.

Data resources

Herbarium of the Institute of Botany, ANAS (BAK)

Dryad Data Repository - doi: 10.5061/dryad.2ngf1vhmw

Results

In total, we examined 24 fresh samples and 14 herbarium vouchers for asterids and 30 fresh samples and six herbarium vouchers for orchids, respectively. We successfully amplified and sequenced all asterids, whereas two collected samples of the orchids dataset did not amplify with any marker and four other samples failed amplification across the three gene regions. Sequence recovery was slightly higher for plastid *rpoB* (88.8% samples) than for ITS (83.3% samples) markers (Tables 1, 2). All herbarium material from both plant lineages was successfully amplified and sequenced with selected barcode markers.

Table 1.

Sequence recovery for the three selected barcode regions from unknown (G1-G38) and Herbarium orchid samples.

Sample	ITS		rpoB		accD	
	amplification	sequencing	amplification	sequencing	amplification	sequencing
G1	x	x	x	x	x	x
G3	x	x	x	x	x	x
G4	x	x	x	x	x	x
G5	x	x	x	x	x	x
G6	x	x	x	x	x	x
G7	x	x	x	x	x	x
G8	NO	NO	x	x	x	NO
G9	x	x	x	x	x	x
G10	x	x	x	x	x	x
G11	x	x	x	x	x	x
G12	x	x	x	x	x	x
G13	NO	NO	x	x	x	NO
G14	x	x	x	x	x	x
G15	x	x	x	x	x	x
G16	x	x	x	x	x	x
G17	x	x	x	x	x	x
G18	x	x	x	x	x	x
G19	x	x	x	x	x	x
G27	x	x	x	x	x	x
G28	NO	NO	NO	NO	NO	NO
G29	x	x	x	x	x	x
G30	NO	NO	NO	NO	NO	NO
G31	x	x	x	x	x	x
G32	x	x	x	x	x	x
G33	NO	NO	x	NO	x	NO
G34	NO	NO	x	NO	x	NO
G35	x	x	x	x	x	x
G36	x	x	x	x	x	x
G37	x	x	x	x	x	x
G38	x	x	x	x	x	x
Orchis purpurea Herbarium 1	x	x	x	x	x	x
Orchis purpurea Herbarium 2	x	x	x	x	x	x

Sample	ITS		rpoB		accD	
	amplification	sequencing	amplification	sequencing	amplification	sequencing
Orchis simia Herbarium 80873	x	x	x	x	x	x
Orchis simia Herbarium 80876	x	x	x	x	x	x
Orchis mascula Herbarium 80801	x	x	x	x	x	x
Orchis mascula Herbarium 80797	x	x	x	x	x	x
	30/36 83.3%		32/36 88.8%		30/36 83.3%	

Table 2.

Sequence recovery for the three selected barcode regions from unknown (P1-A15) and Herbarium asterid samples.

Sample	ITS		rpoB		accD	
	amplification	sequencing	amplification	sequencing	amplification	sequencing
P1	x	x	x	x	x	x
P2	x	x	x	x	x	x
P3	x	x	x	x	x	x
P4	x	x	x	x	x	x
P5	x	x	x	x	x	x
P6	x	x	x	x	x	x
P7	x	x	x	x	x	x
P8	x	x	x	x	x	x
P9	x	x	x	x	x	x
A1	x	x	x	x	x	x
A2	x	x	x	x	x	x
A3	x	x	x	x	x	x
A4	x	x	x	x	x	x
A5	x	x	x	x	x	x
A6	x	x	x	x	x	x
A7	x	x	x	x	x	x
A8	x	x	x	x	x	x
A9	x	x	x	x	x	x
A10	x	x	x	x	x	x
A11	x	x	x	x	x	x
A12	x	x	x	x	x	x
A13	x	x	x	x	x	x
A14	x	x	x	x	x	x

Sample	ITS		rpoB		accD	
	amplification	sequencing	amplification	sequencing	amplification	sequencing
A15	x	x	x	x	x	x
Centaurea trinervia Herbarium 18066	x	x	x	x	x	x
Centaurea trinervia Herbarium 18067	x	x	x	x	x	x
Psephellus hymenolepis Herbarium 22220	x	x	x	x	x	x
Psephellus daghestanicus Herbarium 22262	x	x	x	x	x	x
Psephellus dealbatus Herbarium 22213	x	x	x	x	x	x
Psephellus intergrifolius Herbarium 18082	x	x	x	x	x	x
Psephellus xantocephalus Herbarium 18471	x	x	x	x	x	x
Psephellus transcaucasicus Herbarium 22234	x	x	x	x	x	x
Psephellus transcaucasicus Herbarium 22256	x	x	x	x	x	x
Pyrethrum carneum Herbarium 22357	x	x	x	x	x	x
Taraxacum officinale Herbarium 24510	x	x	x	x	x	x
Senecio vernalis Herbarium	x	x	x	x	x	x
Bellis perennis Herbarium 170015	x	x	x	x	x	x
Centaurea cheiranthifolius Herbarium	x	x	x	x	x	x
	38/38 100%		38/38 100%		38/38 100%	

Local intraspecific variation for plastid barcodes was detected when multiple records were examined. In orchids, more than one haplotype for *accD* were detected in *O. purpurea* and *O. militaris* (Fig. 1) and different haplotypes for *rpoB* were detected in *O. mascula* and *A. pyramidalis* (Fig. 2). ITS base variation was detected in *O. mascula* and an ITS paralogy was detected in *O. purpurea* (Fig. 3).

In asterids, variation for plastid *accD* was detected within genera (*Psephellus*, *Leucanthemum*), but not within species, with the notable exception of two haplotypes found in *Bellis perennis* (Fig. 4). No intraspecific and only very low interspecific variation (i.e. within genera) was detected for *rpoB* (Fig. 5). ITS variation within species was only detected between herbarium and wild-collected *Senecio vernalis* (Fig. 6).
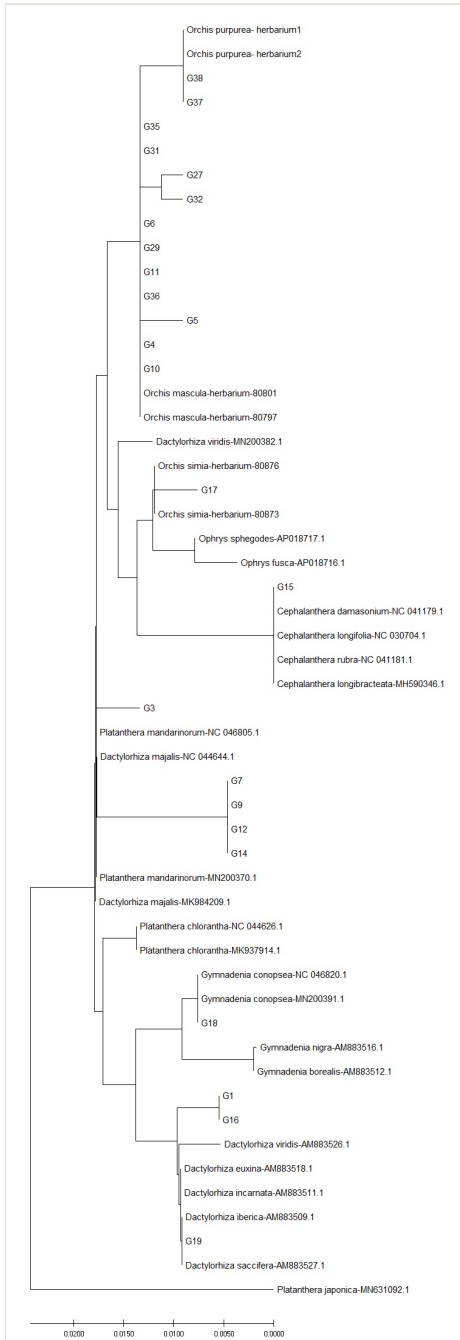
Figure 1. doi

Neighbour-joining phylogenetic tree, based on *accD* sequences of selected orchids. All sequences have been deposited in the Dryad Data Repository - doi: 10.5061/dryad.2ngf1vhmw
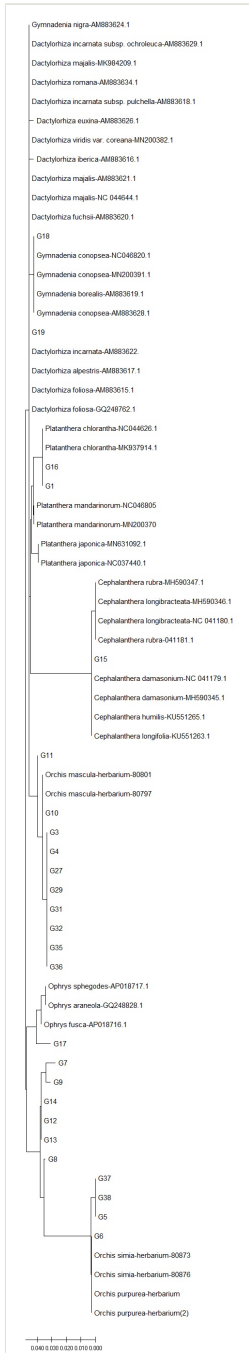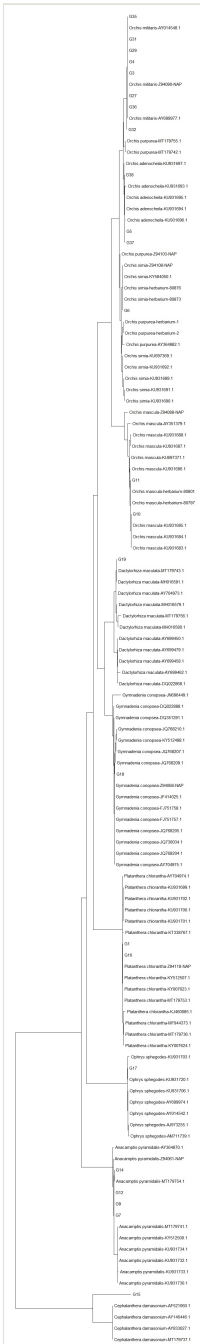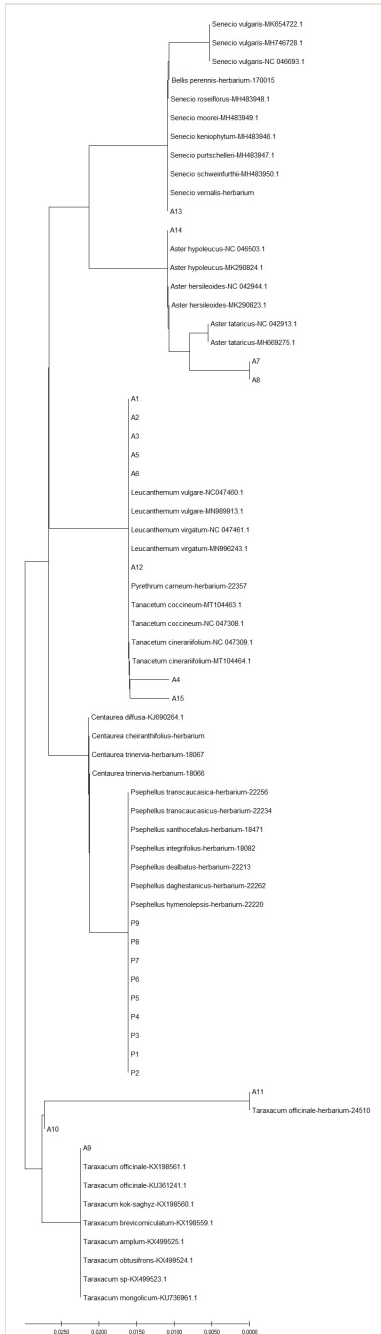
Figure 2. doi

Neighbour-joining phylogenetic tree, based on *rpoB* sequences of selected orchids. All sequences have been deposited in the Dryad Data Repository - doi: 10.5061/dryad.2ngf1vhmw

Figure 3. doi

Neighbour-joining phylogenetic tree, based on ITS sequences of selected orchids. All sequences have been deposited in the Dryad Data Repository - doi: 10.5061/dryad. 2ngf1vhmw

Figure 4. doi
Neighbour-joining phylogenetic tree, based on *accD* sequences of selected asterids. All sequences have been deposited in the Dryad Data Repository - doi: 10.5061/dryad. 2ngf1vhmw
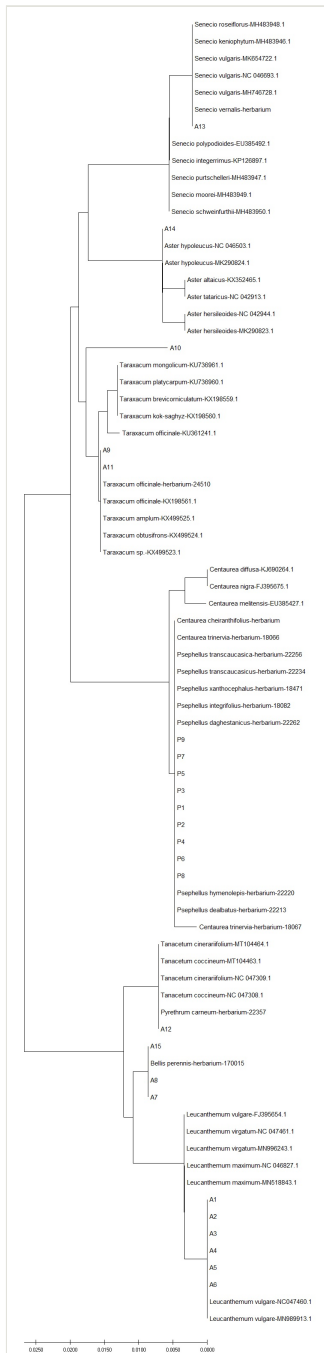
Figure 5. doi

Neighbour-joining phylogenetic tree, based on *rpoB* sequences of selected asterids. All sequences have been deposited in the Dryad Data Repository - doi: 10.5061/dryad. 2ngf1vhmw
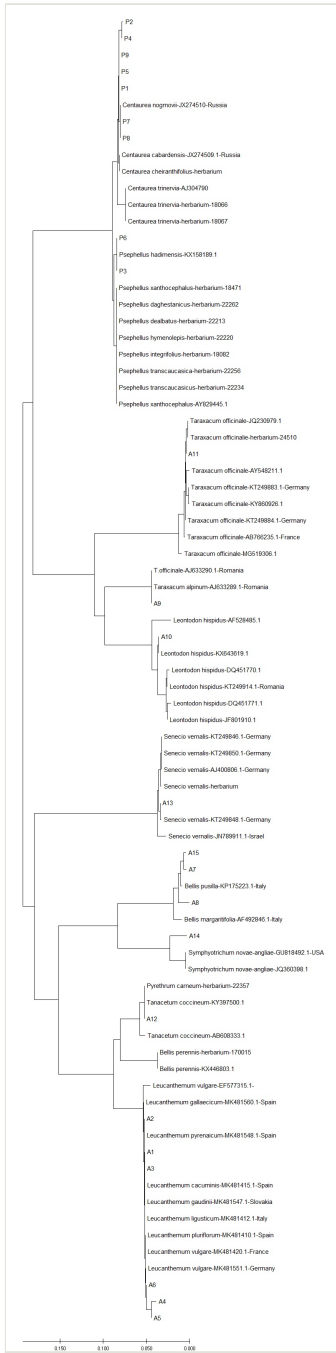
Figure 6. doi

Neighbour-joining phylogenetic tree, based on ITS sequences of selected asterids. All sequences have been deposited in the Dryad Data Repository - doi: 10.5061/dryad. 2ngf1vhmw

Species discrimination ability using BLAST differs for each barcode marker and for the two plant groups. For orchids, ITS provided the highest species resolution (22 out of 26) (Table 3, Suppl. material 1), while both *accD* (1 out of 24) (Table 3, Suppl. material 2) and *rpoB* (3 out of 25) largely failed (Table 3, Suppl. material 3). For asterids, ITS (15 out of 24) (Table 4, Suppl. material 4) and *rpoB* (15 out of 24) (Table 4, Suppl. material 5) gave intermediate values for species resolution, while accD completely failed (Table 4, Suppl. material 6), as most species had identical sequences. Further, for asterids, there were several discrepancies on species assignment depending on the employed marker even when the top Bit-Score obtained in the GenBank matched a single species (in bold in Table 4).

Table 3.

Orchid species resolution for each barcode region, based on an all-to-all Blast analysis.

NO[1]: more than one reference sequence at top Bit-Score (at least 99.5%)

NO[2]: all reference sequences at top Bit-score lower than 99.5%

Sample	ITS	accD	rpoB
G1	NO[1]	NO[1]	(*Platanthera chlorantha*)
G3	(*Orchis militaris*)	NO[1]	NO [2]
G4	(*Orchis militaris*)	NO[1]	NO [2]
G5	(*Orchis adenocheila*)	NO[2]	NO [2]
G6	(*Orchis simia*)	NO[1]	NO [2]
G7	(*Anacamptis pyramidalis*)	NO[2]	NO [2]
G8			NO [2]
G9	(*Anacamptis pyramidalis*)	NO[2]	NO[2]
G10	(*Orchis mascula*)	NO [1]	NO[2]
G11	(*Orchis mascula*)	NO [1]	NO[2]
G12	(*Anacamptis pyramidalis*)	NO[2]	NO[2]
G13			NO [2]
G14	(*Anacamptis pyramidalis*)	NO[2]	NO[2]
G15	NO[1]	NO[1]	NO[1]
G16	NO[1]	NO[1]	(*Platanthera chlorantha*)
G17	(*Ophrys sphegodes*)	NO[2]	NO[2]
G18	(*Gymnadenia conopsea*)	NO[1]	(*Gymnadenia conopsea*)
G19	NO[1]	(*Dactylorhiza saccifera*)	NO[1]
G27	(*Orchis militaris*)	NO[2]	NO[2]
G29	(*Orchis militaris*)	NO[1]	NO [2]
G31	(*Orchis militaris*)	NO[2]	NO[2]
G32	(*Orchis militaris*)	NO[2]	NO[2]
G35	(*Orchis militaris*)	NO[2]	NO[2]

Sample	ITS	accD	rpoB
G36	(*Orchis militaris*)	NO[1]	NO [2]
G37	(*Orchis adenocheila*)	NO[2]	NO[2]
G38	(*Orchis adenocheila*)	NO[2]	NO[2]

Table 4.

Asterid species resolution for each barcode region, based on an all-to-all Blast analysis.

NO[1]: more than one reference sequence at top Bit-Score (at least 99.5%)

NO[2]: all reference sequences at top Bit-score lower than 99.5%

Sample	ITS	accD	rpoB
P1	(***Centaurea nogmovii***)	NO [1]	(***Carthamus tinctorius***)
P2	NO [2]	NO [1]	(*Carthamus tinctorius*)
P3	(***Psephellus hadimensis***)	NO [1]	(***Carthamus tinctorius***)
P4	(***Centaurea nogmovii***)	NO [1]	(***Carthamus tinctorius***)
P5	(***Centaurea nogmovii***)	NO [1]	(***Carthamus tinctorius***)
P6	(***Psephellus hadimensis***)	NO [1]	(***Carthamus tinctorius***)
P7	(***Centaurea nogmovii***)	NO [1]	(***Carthamus tinctorius***)
P8	(***Centaurea nogmovii***)	NO [1]	(***Carthamus tinctorius***)
P9	(***Centaurea nogmovii***)	NO [1]	(***Carthamus tinctorius***)
A1	NO[1]	NO [1]	(*Leucanthemum vulgare*)
A2	NO [2]	NO [1]	(*Leucanthemum vulgare*)
A3	NO [1]	NO [1]	(*Leucanthemum vulgare*)
A4	NO[2]	NO [1]	(*Leucanthemum vulgare*)
A5	NO[1]	NO [1]	(*Leucanthemum vulgare*)
A6	NO [2]	NO [1]	(*Leucanthemum vulgare*)
A7	(*Bellis pusilla*)	NO [2]	NO[1]
A8	(*Bellis pusilla*)	NO [2]	NO[1]
A9	(*Hypochaeris radicata*)	NO[1]	NO[1]
A10	(*Leontodon hispidus*)	NO[1]	NO[1]
A11	NO [1]	NO [2]	NO[1]
A12	(*Tanacetum coccineum*)	NO[1]	NO[1]
A13	(*Senecio vernalis*)	NO[1]	NO[1]
A14	(*Symphyotrichum novae-angliae*)	NO[1]	NO[1]
A15	NO[2]	NO[1]	NO[1]

Table 5.

Orchid species resolution for each barcode region, based on the NJ tree (i.e. monophyletic species)

Sample	ITS	accD	rpoB
G1	(*Platanthera chlorantha*)	NO	(*Platanthera chlorantha*)
G3	(*Orchis militaris*)	NO	NO
G4	(*Orchis militaris*)	NO	NO
G5	(*Orchis adenocheila*)	NO	NO
G6	(*Orchis simia*)	NO	NO
G7	(*Anacamptis pyramidalis*)	NO	NO
G9	(*Anacamptis pyramidalis*)	NO	NO
G10	(*Orchis mascula*)	NO	(*Orchis mascula*)
G11	(*Orchis mascula*)	NO	NO
G12	(*Anacamptis pyramidalis*)	NO	NO
G14	(*Anacamptis pyramidalis*)	NO	NO
G15	NO (*Cephalanthera* sp.)	NO (*Cephalanthera* sp.)	NO (*Cephalanthera* sp.)
G16	(*Platanthera chlorantha*)	NO	(*Platanthera chlorantha*)
G17	(*Ophrys sphegodes*)	(*Orchis simia*)	NO (*Ophrys* sp.)
G18	(*Gymnadenia conopsea*)	(*Gymnadenia conopsea*)	(*Gymnadenia conopsea*)
G19	(*Dactylorhiza maculata*)	NO (*Dactylorhiza* sp.)	NO (*Dactylorhiza* sp.)
G27	*Orchis militaris*	NO	NO
G29	(*Orchis militaris*)	NO	NO
G31	(*Orchis militaris*)	NO	NO
G32	(*Orchis militaris*)	NO	NO
G35	(*Orchis militaris*)	NO	NO
G36	(*Orchis militaris*)	NO	NO
G37	(*Orchis adenocheila*)	(*Orchis purpurea*)	NO
G38	(*Orchis adenocheila*)	(*Orchis purpurea*)	NO
G13			NO
G8			NO

Table 6.

Asterid species resolution for each barcode region, based on the NJ tree (i.e. monophyletic species).

Sample	ITS	accD	rpoB
P1	NO (*Centaurea* sp.)	NO (*Psephellus* sp.)	NO (*Psephellus* sp.)
P2	NO	NO (*Psephellus* sp.)	NO (*Psephellus* sp.)
P3	(*Psephellus hadimensis*)	NO (*Psephellus* sp.)	NO (*Psephellus* sp.)
P4	NO (*Centaurea* sp.)	NO (*Psephellus* sp.)	NO (*Psephellus* sp.)
P5	NO (*Centaurea* sp.)	NO (*Psephellus* sp.)	NO (*Psephellus* sp.)
P6	(*Psephellus hadimensis*)	NO (*Psephellus* sp.)	NO (*Psephellus* sp.)
P7	(*Centaurea nogmovii*)	NO (*Psephellus* sp.)	NO (*Psephellus* sp.)
P8	(*Centaurea nogmovii*)	NO (*Psephellus* sp.)	NO (*Psephellus* sp.)
P9	NO (*Centaurea* sp.)	NO (*Psephellus* sp.)	NO (*Psephellus* sp.)
A1	NO (*Leucanthemum* sp.)	NO (*Leucanthemum* sp.)	(*Leucanthemum vulgare*)
A2	NO (*Leucanthemum* sp.)	NO (*Leucanthemum* sp.)	(*Leucanthemum vulgare*)
A3	NO (*Leucanthemum* sp.)	NO (*Leucanthemum* sp.)	(*Leucanthemum vulgare*)
A4	NO	NO	(*Leucanthemum vulgare*)
A5	NO	NO (*Leucanthemum* sp.)	(*Leucanthemum vulgare*)
A6	NO (*Leucanthemum* sp.)	NO (*Leucanthemum* sp.)	(*Leucanthemum vulgare*)
A7	(*Bellis pusilla*)	NO	(*Bellis perennis*)
A8	NO (*Bellis* sp.)	NO	(*Bellis perennis*)
A9	NO (*Taraxacum* sp.)	NO (*Taraxacum* sp.)	NO (*Taraxacum* sp.)
A10	(*Leontodon hispidus*)	NO	NO
A11	(*Taraxacum officinale*)	(*Taraxacum Officinale*)	NO (*Taraxacum* sp.)
A12	(*Tanacetum coccineum*)	NO	NO
A13	(*Senecio vernalis*)	NO (*Senecio* sp.)	NO (*Senecio* sp.)
A14	NO	NO (*Aster* sp.)	(*Aster hypoleucus*)
A15	NO	NO	(*Bellis perennis*)

ITS showed the highest discriminatory power also when evaluating genetic distances within and between species by NJ tree. This was evident in orchids: more than 90% of the sequences collected in this study had inter-specific diversity higher than intra-specific diversity, indicating that the ITS sequences had clear species boundaries and all accessions of the same species clustered in a monophyletic group (Table 5). Instead, in asterids, the discriminatory power of ITS marker was higher when discriminating amongst genera, but comparable with plastid markers when referring to species assignment (Table 6). When geographic origins of Genbank available sequences were plotted on the NJ tree, the ITS marker showed the phylogeographic signal for orchids (Fig. 7, Suppl. material 7),

less for asterids (Fig. 8, Suppl. material 8). No phylogeographic variation was detected with plastid barcodes (data not shown).

Discussion

We have tested the potential of barcode markers on a selection of herbal groups that are traditionally difficult to be morphologically identified since discriminant flower traits are not always available. Typically, a species discrimination is successful when the following conditions are met: i) all individual barcode sequences are not shared by any other species in the dataset; ii) genetic variation within species is lower than amongst species (i.e. the barcoding gap); iii) all individuals of a species cluster together in a monophyletic group when employing distance-based neighbour-joining (NJ) tree, at least at a local scale. Preliminary analyses of available information in public databases (GenBank) and literature data (Jin et al. 2014, Gao et al. 2010) confirmed the low level of species resolution when using traditional *rbcL* and *matK* barcodes in these two selected herbal groups. For this reason, we preferred testing complementary barcode markers, such as chloroplast *rpoB*, *accD* and nuclear ITS that are expected to have higher discriminatory power, particularly in annual/rapidly evolving herbaceous groups as the ones we were focused on (Chen et al. 2010, Gao et al. 2010). We chose these barcodes because of the sequence availability in public databases or, in the absence of available sequences, because of the level of interspecific variability detected with the same markers in related plant groups (Gigot et al. 2007, Dong et al. 2012).

We found that the selected barcodes successfully amplified and sequenced all asterids and almost all orchids (likely depending on the quality of dried samples, i.e. orchids have thicker leaves than asterids), but we found that the rate of species-level resolution largely varies amongst selected markers and plant groups. Overall, for both plant lineages, plastid markers had a species discrimination success rate lower than nuclear ITS, which allowed us, at least for orchids, to univocally discriminate most species. Sequence accessions of each species clustered together in monophyletic groups confirming the existence of a barcoding gap (Fig. 3). As already found in previous studies (Aceto et al. 1999, Cozzolino et al. 2001), variation found in the ITS region allows determination of genetic divergence amongst orchid species.

In orchids, ITS demonstrated a higher successful discrimination capability compared to both plastid markers, whereas in asterids, both ITS and *rpoB* had a comparable identification success (Table 4). *accD* completely fails in identifying asterids and most of orchids for both BLAST and the nearest genetic distance method. The lower identification success of plastid markers (particularly of *accD*) is largely due to its low discriminatory power (different species with identical sequences) or because of missing available reference sequences (Suppl. materials 2, 1, 3, 4, 5, 6, 7, 8).

Figure 7. doi

Neighbour-joining phylogenetic tree, based on *ITS* sequences of selected orchids with geographic origins (green: Europe; red: Asia) as inferred from Suppl. material 7.
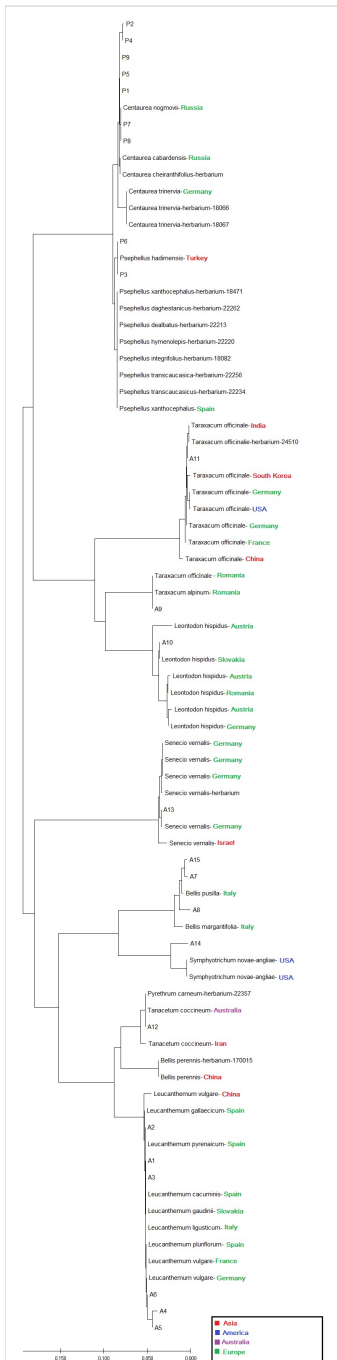
Figure 8. doi

Neighbour-joining phylogenetic tree based, on *ITS* sequences of selected asterids with geographic origins (green: Europe; red: Asia) as inferred from Suppl. material 8.

In asterids, we also detected a discrepancy between species assignment with the query sequences (i.e. at least 99.5% of identical sites to reference sequences) for different barcodes. An example is given by the accession P6: ITS marker shared a top Bit-Score (100%) with the *Psephellus hadimensis* reference sequence, while the *rpoB* marker shared a top Bit-Score (100%) with the *Carthamus tinctorius* reference sequence (Table 4). Indeed, efficiency evaluation of correct assignment with DNA barcoding markers depends both on how informative are the generated sequences and how many sequences of representative groups are already available in public DNA databases. Ideally, the accuracy of specimen identification is highly dependent on representation of databases in which target species are represented by several individuals (Meyer and Paulay 2005) from different geographic origins. However, such databases are often not sufficiently complete and suited to exclude the risk of sequence matching due to missing data or of incorrect estimation of the barcoding gap (Meyer and Paulay 2005), as we found here, particularly for our plastid markers. In this perspective, the combined use of both unknown material and well-identified herbarium specimens, as implemented in our study, may partially fulfil such weaknesses (Kuzmina et al. 2017). Nevertheless, in case of discrepancy in the species assignment with different DNA barcoding markers, we preferred the assignment, based on those markers with larger bulk of reference sequences and/or that allow accessions to cluster monophyletically with distance-based approaches.

The discreteness of species boundaries, particularly in hybridising and/or fast-radiating lineages, may reduce the discriminatory power of barcode markers (Chase and Fay 2009, Kress et al. 2009). For this reason, the combined use of plastid and nuclear markers allows testing for hybridisation/reticulate evolution. In our case, we only detected a single case of ITS paralogy (in *O. purpurea*). Overall, we did not detect a discordantspecies relationship depending on the used barcode (nuclear or plastid) that could be a clear indication of hybridisation/reticulate evolution. This points to the low plastid marker resolution amongst closely-related species more likely due to their recent radiation (particularly in asterids). In that case, if barcode markers are evolving slowly, relative to the speciation rate, there may be insufficient nucleotide differences to distinguish recent species (Fazekas et al. 2009).

Barcode markers that univocally allow identifying species can also be used to reconstruct main phylogeographic patterns, if they contain enough intra-specific variability. In such cases, comparison of barcode sequences of plant specimens collected throughout their geographical ranges may provide sufficient informative data for allocating individuals to a well-defined geographic origin. Here, we also estimated whether nuclear and plastid markers were sufficiently variable to provide insights into the historical phylogeography and to detect the pattern of geographical distribution of infraspecific variation in Caucasian orchids and asterids. In our case, both plastid markers almost fail in identifying geographic origins of orchid and asterid accessions of different origins (identical barcode sequences) while ITS, at least for orchids, displayed enough infraspecific variation leading to different geographic rybotypes, potentially useful for tracking origins of plant materials.

Terrestrial orchids occurred both in the Caucasus and Europe. In particular, terrestrial Orchidinae probably originate from Irano-Turanian and Caucasus elements (the Irano-Turanian and Caucasus origin) and came into the Mediterranean basin during the

Messinian age where their radiation gave rise to one of the richest systems of vicariant endemism between the two floristic regions. Some Mediterranean radiated lineages have then secondarily recolonised the Caucasian region (Batemann et al. 2003). Interestingly, for some orchid species (*Orchis mascula, Platanthera chloranta, Anacamptis pyramidalis*; Fig. 8), ITS sequences clearly display such geographic variation (from west to east and vice versa), while, for other species, almost no sequence variation occurs across all ranges (*Orchis militaris)*. In the former case, we suggest that this intraspecific variation represents the signature of ancient phylogeographic routes, whereas in the latter, with no intraspecific variation, we suspect recent post glacial phylogeographic migration erased the ancient phylogeographic signal.

Conclusions

We found, for both lineages, plastid markers had a species-level assignment success rate lower that nuclear ITS marker. Several processes, such as recent speciation events with incomplete lineage sorting and retention of ancestral sequences, may cause a partial failure of DNA barcodes to track species events. Indeed, the ITS sequence was successful in orchids, but not in many asterids. We argue that, at least between the two herbal groups, the diversification time marked the difference in barcode efficiency as the absence of a barcoding gap amongst closely-related, recently-diverged species is quite common. While orchids represent an old evolutionary lineage, with some groups radiating in the Mediterranean and secondarily migrating to the Caucasus (Batemann et al. 2003), diversification of asterid lineages is more recent and had its centre in the Caucasus and surrounding west Asia (Barres et al. 2013). In contrast to orchids, many closely-related asterids species occur within a geographically-restricted context, which makes difficult their discrimination, particularly with plastid barcodes. Overall, our study suggests that the ITS sequence can be potentially utilised as universal plant barcodes in herbal groups; at the same time, it highlights that ITS sequence efficiency as barcode marker and its discriminatory power are strongly dependent on the evolutionary history of the examined plant group.

Acknowledgements

Author contributions

Salvatore Cozzolino and Donata Cafasso performed the DNA extraction, all the bioinformatics analyses and the molecular characterisation of the identified plants. Parvin Aghayeva collected and provided the plant samples. Salvatore Cozzolino, Silvia Fineschi, Dilzara Aghayeva and Valida Ali-zade supervised the work, provided funding and edited the manuscript.

Conflicts of interest

None

References

- Aceto S, Caputo P, Cozzolino S, Gaudio L, Moretti A (1999) Phylogeny and evolution of *Orchis* and allied genera based on ITS DNA variation: morphological gaps and molecular continuity. Molecular Phylogenetics and Evolution 13 (1): 67-76. https://doi.org/10.1006/mpev.1999.0628
- Aghayeva P, Qarakhani P, Huseynova A, Alizade V (2018) Wild ornamental plants of the family Asteraceae from the northeastern part of Azerbaijan. Chornomors'k Botanical Journal 14 (3): 204-212. https://doi.org/10.14255/2308-9628/18.143/1
- Alizade V, Mehdiyeva N, Karimov V, Ibrahimova A (2019) Plants of the Greater Caucasus. Red N Line Publ. House, Baku, 352 pp.
- Barres L, Sanmartín I, Anderson C, Susanna A, Buerki S, Galbany-Casals M, Vilatersana R (2013) Reconstructing the evolution and biogeographic history of tribe Cardueae (Compositae). American Journal of Botany 100 (5): 867-882. https://doi.org/10.3732/ajb.1200058
- Batemann R, Hollingsworth P, Preston J, Yi-Bo L, Pridgeon A, Chase M (2003) Molecular phylogenetics and evolution of Orchidinae and selected Habenariinae (Orchidaceae). Botanical Journal of the Linnean Society 142 (1): 1-40. https://doi.org/10.1046/j.1095-8339.2003.00157.x
- Bohn U, Zazanashvili N, Nakhutsrishvili G (2007) The map of the natural vegetation of Europe and its application in the Caucasus ecoregion. Bulletin of the Georgia Academy of Science 175 (1): 112-121. URL: http://science.org.ge/old/moambe/2007-vol1/112-120.pdf
- Burgess K, Fazekas A, Kesanakurti R, Graham SW, Husband B, Newmaster S, Percy D, Hajibabaei M, Barrett S (2011) Discriminating plant species in a local temperate flora using the rbcL+matK DNA barcode. Methods in Ecology and Evolution 2 (4): 333-340. https://doi.org/10.1111/j.2041-210x.2011.00092.x
- Castro C, Hernandez A, Alvarado L, Flores D (2015) DNA barcodes in fig cultivars (*Ficus carica* L.) using ITS regions of ribosomal DNA, the psbA-trnH spacer and the matK coding equence. American Journal of Plant Sciences 06 (01): 95-102. https://doi.org/10.4236/ajps.2015.61011
- Chase M, Fay M (2009) Barcoding of plants and fungi. Science 325 (5941): 682-683. https://doi.org/10.1126/science.1176906
- Chen S, Yao H, Han J, Liu C, Song J, Shi L, Zhu Y, Ma X, Gao T, Pang X, Luo K, Li Y, Li X, Jia X, Lin Y, Leon C (2010) Validation of the ITS2 region as a novel DNA barcode for identifying medicinal plant species. PLOS One 5 (1). https://doi.org/10.1371/journal.pone.0008613
- Costion C, Lowe A, Rossetto M, Kooyman R, Breed M, Ford A, Crayn D (2016) Building a Plant DNA Barcode Reference Library for a Diverse Tropical Flora: An Example from Queensland, Australia. Diversity 8 (1): 1. https://doi.org/10.3390/d8010005
- Cozzolino S, Caputo P, Aceto S, Widmer A, Dafni A (2001) Speciation processes in Eastern Mediterranean *Orchis* s.l. species: Molecular evidence and the role of

pollination biology. Israel Journal of Plant Sciences 49 (2): 91-103. https://doi.org/10.1560/qv6m-e7a0-qfc7-g6bl

- Dong W, Liu J, Yu J, Wang L, Zhou S (2012) Highly variable chloroplast markers for evaluating plant phylogeny at low taxonomic levels and for DNA barcoding. PLOS One 7 (4). https://doi.org/10.1371/journal.pone.0035071

- Ebihara A, Nitta J, Ito M (2010) Molecular species identification with rich floristic sampling: DNA barcoding the Pteridophyte flora of Japan. PLOS One 5 (12). https://doi.org/10.1371/journal.pone.0015136

- Fazekas A, Kesanakurt P, Burgess K, Percy D, Graham S, Barrett S, Newmaster S, Hajibabaei M, Husband B (2009) Are plant species inherently harder to discriminate than animal species using DNA barcoding markers? Molecular Ecology Resources 9: 130-139. https://doi.org/10.1111/j.1755-0998.2009.02652.x

- Feng S, Jiang M, Shi Y, Jiao K, Shen C, Lu J, Ying Q, Wang H (2016) Application of the ribosomal DNA ITS2 region of *Physalis* (Solanaceae): DNA barcoding and phylogenetic tudy. Frontiers in Plant Science 7 (1047): 1-11. https://doi.org/10.3389/fpls.2016.01047

- Galimberti A, Casiraghi M, Bruni I, Guzzetti L, Cortis P, Berterame NM, Labra M (2019) From DNA barcoding to personalized nutrition: the evolution of food traceability. Current Opinion in Food Science 28: 41-48. https://doi.org/10.1016/j.cofs.2019.07.008

- Gao T, Yao H, Song J, Zhu Y, Liu C, Chen S (2010) Evaluating the feasibility of using candidate DNA barcodes in discriminating species of the large Asteraceae family. BMC Evolutionary Biology 10 (1). https://doi.org/10.1186/1471-2148-10-324

- Gigot G, Van Alphen-Stahl J, Bogarín D, Warner J, Chase M, Savolainen V (2007) Finding a suitable DNA barcode for Mesoamerican orchids. Lankesteriana 7 (1-2): 200-203. https://doi.org/10.15517/lank.v7i1-2.19502

- Gray S, Brady S (2016) Plant developmental responses to climate change. Developmental Biology 419 (1): 64-77. https://doi.org/10.1016/j.ydbio.2016.07.023

- Grossheim A (1949) Определитель растений Кавказа. Москва: Советская наука. 1949. 747с. [Key to plants of the Caucasus]. Soviet Science, Moscow, 747 pp. [In Russian].

- Hall T (2018) BioEdit: An important software for molecular biology. GERF Bulletin of Biosciences 2 (1). [In English].

- Hebert P, Penton E, Burns J, Janzen D, Hallwachs W (2004) Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly *Astraptes fulgerator*. Proceedings of the National Academy of Sciences 101 (41): 14812-14817. https://doi.org/10.1073/pnas.0406166101

- Heywood V (2003) Conservation and sustainable use of wild species as sources of new ornamentals. Acta Horticulturae 598: 43-53. https://doi.org/10.17660/actahortic.2003.598.5

- Hollingsworth P, Graham S, Little D (2011) Choosing and using a plant DNA Barcode. PLOS One 6 (5): e19254. https://doi.org/10.1371/journal.pone.0019254

- Hosseinzadeh-Colagar A, Haghighatnia M, Amiri Z, Mohadjerani M, Tafrihi M (2016a) Microsatellite (SSR) amplification by PCR usually led to polymorphic bands: Evidence which shows replication slippage occurs in extendor nascent DNA strands. Molecular Biology Research Communications 5 (3): 167-174. URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5219911/pdf/mbrc-5-167.pdf

- Hosseinzadeh-Colagar A, Yousefzadeh H, Shayanmehr F, Jalali SG, Zare H, Tippery N (2016b) Molecular taxonomy of Hyrcanian *Alnus* using nuclear ribosomal ITS and

chloroplast trnH-psbA DNA barcode markers. Systematics and Biodiversity 14 (1): 88-101. https://doi.org/10.1080/14772000.2015.1102172

- Hubert N, Hanner R (2015) DNA Barcoding, species delineation and taxonomy: a historical perspective. DNA Barcodes 3 (1). https://doi.org/10.1515/dna-2015-0006

- Huemer P, Hebert P (2011) Cryptic diversity and phylogeography of high alpine *Sattleria* —a case study combining DNA barcodes and morphology (Lepidoptera: Gelechiidae). Zootaxa 2981 (1): 1-22. https://doi.org/10.11646/zootaxa.2981.1.1

- Hu H, Al-Shehbaz I, Sun Y, Hao G, Wang Q, Liu J (2015) Species delimitation in *Orychophragmus* (Brassicaceae) based on chloroplast and nuclear DNA barcodes. Taxon 64 (4): 714-726. https://doi.org/10.12705/644.4

- Jin W, Jin X, Schuiteman A, Li D, Xiang X, Huang W, Li JW, Huang L (2014) Molecular systematics of subtribe Orchidinae and Asian taxa of Habenariinae (Orchideae, Orchidaceae) based on plastid matK, rbcL and nuclear ITS. Molecular Phylogenetics and Evolution 77 https://doi.org/10.1016/j.ympev.2014.04.004

- Karjagin I (1928) Materials on the vegetation of the Quba region [Материалы по растительности Кубинского уезда]. Proceedings of Azerbaijan State University 7: 121-141. [In Russian].

- Karjagin I (Ed.) (1950) Флора Азербайджана. [Flora of Azerbaijan]. Vol. I-VIII. Publishing house of Academy of Sciences of Azerb. SSR, Baku. [In Russian].

- Kress W, Erickson D, Jones F, Swenson N, Perez R, Sanjur O, Bermingham E (2009) Plant DNA barcodes and a community phylogeny of a tropical forest dynamics plot in Panama. Proceedings of the National Academy of Sciences 106 (44): 18621-18626. https://doi.org/10.1073/pnas.0909820106

- Kuzmina M, Braukmann T, Fazekas A, Graham S, Dewaard S, Rodrigues A, Bennett B, Dickinson T, Saarela J, Catling P, Newmaster S, Percy D, Fenneman E, Lauron-Moreau A, Ford B, Gillespie L, Subramanyam R, Whitton J, Jennings L, Metsger D, Warne C, Brown A, Sears E, Dewaard J, Zakharov E, Hebert P (2017) Using herbarium-derived DNAs to assemble a large-scale DNA Barcode Library for the vascular plants of Canada. Applications in Plant Sciences 5 (12). https://doi.org/10.3732/apps.1700079

- Li D, Gao L, Li H, Wang H, Ge X, Yang J, Liu J, Chen Z, Zhou S, Chen S, Jang J, Fu C, Zeng C, Yan H, YJ Z, Sun Y, Zhao, Wang K, Yang T, Duan G (2011) Comparative analysis of a large dataset indicates that internal transcribed spacer (ITS) should be incorporated into the core barcode for seed plants. Proceedings of the National Academy of Sciences 108 (49): 19641-19646. https://doi.org/10.1073/pnas.1104551108

- Meyer C, Paulay G (2005) DNA Barcoding: Error rates based on comprehensive sampling. PLOS Biology 3 (12): 2229-2238. https://doi.org/10.1371/journal.pbio.0030422

- Moore W (1995) Inferring phylogenies from mtDNA variation: mitochondrial-gene trees versus nuclear-gene trees. Evolution 49 (4): 718-726. https://doi.org/10.2307/2410325

- Saravanan M, Mohanapriya G, Laha R, Sathishkumar R (2019) DNA barcoding detects floral origin of Indian honey samples. Genome 62 (5): 341-348. https://doi.org/10.1139/gen-2018-0058

- Tutayuk V, Gadzhiev V, Vagabov Z (1961) Wild growing ornamental plants in the mountains of the Greater Caucasus [Тутаюк В.Х., Гаджиев В.Д., Вагабов З.В. (1961) Дикорастущие декаративные растений в горах Большого Кавказа. Известия АН Азерб.ССР, сер.биол. и медиц.наук, 8: 3-13.]. Proceedings of the Academy of Sciences of the Azerbaijan SSR, ser.biol. and medical science 8 (1): 3-13. [In Russian].

- Valentini A, Pompanon F, Taberlet P (2009) DNA barcoding for ecologists. Trends in Ecology & Evolution 24 (2): 110-117. https://doi.org/10.1016/j.tree.2008.09.011
- Vences M, Thomas M, van der Meijden A, Chiari Y, Vieites DR (2005) Comparative performance of the 16SrRNA gene in DNA barcoding of amphibians. Frontiers in Zoology 2 (5). URL: https://frontiersinzoology.biomedcentral.com/articles/10.1186/1742-9994-2-5
- Vijayan K, Tsou C (2010) DNA barcoding in plants: taxonomy in a new perspective. Current Science 99 (11): 1530-1541. URL: https://www.currentscience.ac.in/Downloads/article_id_099_11_1530_1541_0.pdf
- Wells J, Stevens J (2008) Application of DNA-based methods in forensic entomology. Annual Review of Entomology 53: 103-12. https://doi.org/10.1146/annurev.ento.52.110405.091423
- Xu L, Gong W, Cusack S, Wu H, Loovers H, Zhang H, Perrett S, Jones G (2018) The β6/β7 region of the Hsp70 substrate-binding domain mediates heat-shock response and prion propagation. Cellular and Molecular Life Sciences 75 (8): 1445-1459. https://doi.org/10.1007/s00018-017-2698-3

Supplementary materials

Suppl. material 1: Results of BLAST species identification test for orchid ITS `doi`

Authors: Parvin Aghayeva, Salvatore Cozzolino, Donata Cafasso, Valida Ali-zade, Silvia Fineschi, Dilzara Aghayeva
Data type: Top Bit-score
Brief description: BLAST identification for orchid
Download file (16.33 kb)

Suppl. material 2: Results of BLAST species identification test for orchid *accD* `doi`

Authors: Parvin Aghayeva, Salvatore Cozzolino, Donata Cafasso, Valida Ali-zade, Silvia Fineschi, Dilzara Aghayeva
Data type: Top Bit-score
Brief description: BLAST identification for orchid
Download file (15.50 kb)

Suppl. material 3: Results of BLAST species identification test for orchid *rpoB* `doi`

Authors: Parvin Aghayeva, Salvatore Cozzolino, Donata Cafasso, Valida Ali-zade, Silvia Fineschi, Dilzara Aghayeva
Data type: Top Bit-score
Brief description: BLAST identification for orchid
Download file (15.39 kb)

Suppl. material 4: Results of BLAST species identification test for asterid ITS `doi`

Authors: Parvin Aghayeva, Salvatore Cozzolino, Donata Cafasso, Valida Ali-zade, Silvia Fineschi, Dilzara Aghayeva
Data type: Top Bit-score

Brief description: BLAST identification for asterid
Download file (14.31 kb)

Suppl. material 5: Results of BLAST species identification test for asterid *rpoB* `doi`

Authors: Parvin Aghayeva, Salvatore Cozzolino, Donata Cafasso, Valida Ali-zade, Silvia Fineschi, Dilzara Aghayeva
Data type: Top Bit-score
Brief description: BLAST identification for asterid
Download file (15.39 kb)

Suppl. material 6: Results of BLAST species identification test for asterid *accD* `doi`

Authors: Parvin Aghayeva, Salvatore Cozzolino, Donata Cafasso, Valida Ali-zade, Silvia Fineschi, Dilzara Aghayeva
Data type: Top Bit-score
Brief description: BLAST identification for asterid
Download file (25.25 kb)

Suppl. material 7: Highest BLAST match of ITS sequences for the samples of Orchidaceae examined in this study `doi`

Authors: Parvin Aghayeva, Salvatore Cozzolino, Donata Cafasso, Valida Ali-zade, Silvia Fineschi, Dilzara Aghayeva
Data type: BLAST match
Brief description: BLAST match of ITS sequences for Orchidaceae
Download file (293.50 kb)

Suppl. material 8: Highest BLAST match of ITS sequences for the samples of Asteraceae examined in this study `doi`

Authors: Parvin Aghayeva, Salvatore Cozzolino, Donata Cafasso, Valida Ali-zade, Silvia Fineschi, Dilzara Aghayeva
Data type: BLAST match
Brief description: BLAST match of ITS of Asteraceae
Download file (191.75 kb)