Article

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The genus *Iris* as a critical taxon in establishing an integrated approach to Italian plant biodiversity

Abstract

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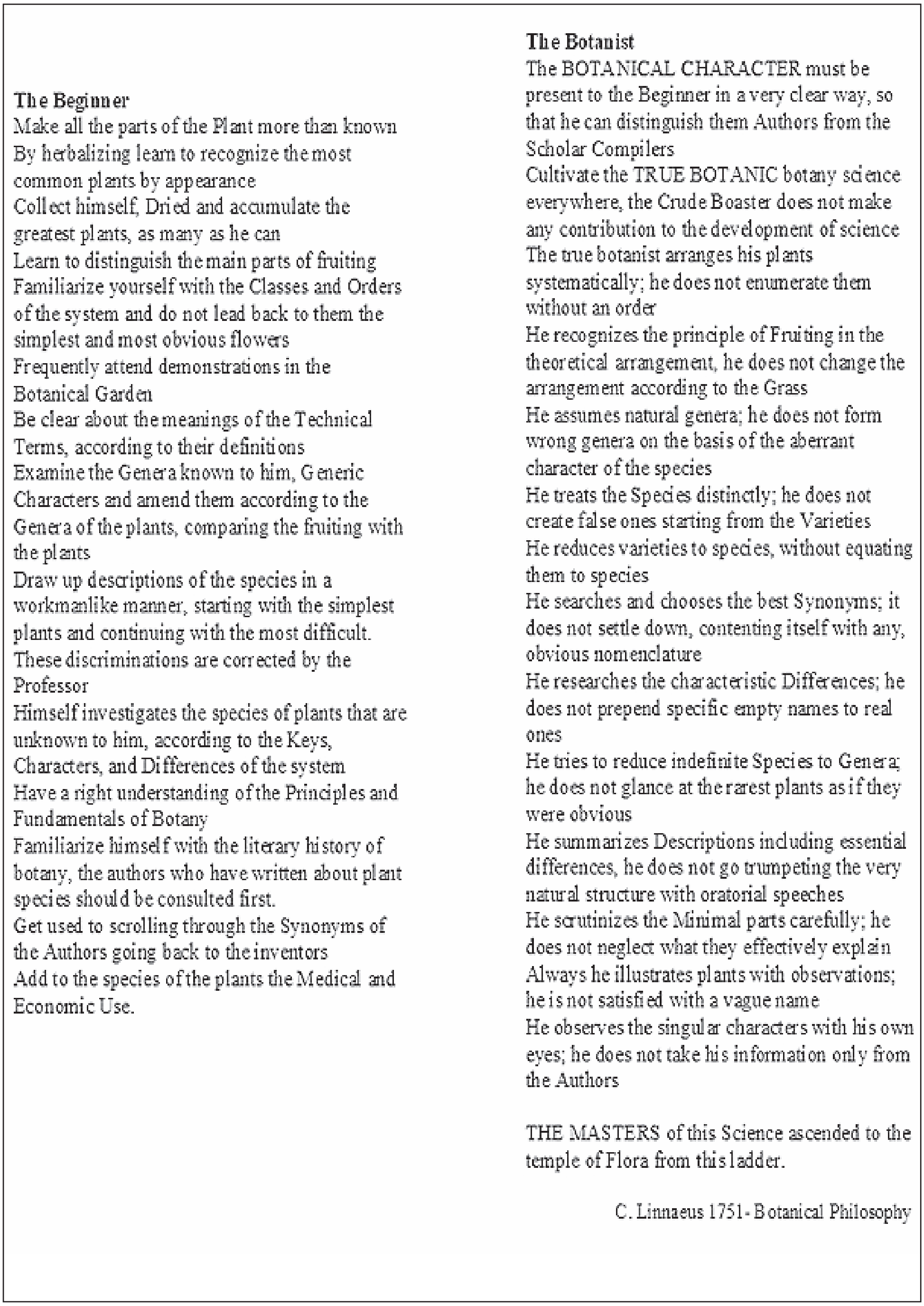
Accurate updating of systematic information for protection and preservation of plant biodiversity can encounter many problems, both in floristics and in the context of other appropriate and useful technologies. We highlight ongoing studies on the complex genus *Iris* Tourn. ex L. in Italy as a case-study to stimulate researchers into providing new data for the understanding and conservation of natural biodiversity. *Iris* is species-rich, with 300 species and many hybrids, allowing a wide range of suitable techniques and methods, including experimental crosses. We provide examples in which dissemination of hasty, conflicting, misleading or erroneous information through the literature and inaccurate websites can be filtered and validated. Careful initial identification of the taxa and a synergistic biosystematic investigation can help to improve effective quantification and qualification of biodiversity among experts and nature lovers.

Key words: hybrids, phylogeny, plant identification, species investigation, threatened species.

Introduction

To protect and preserve plant biodiversity, it is necessary to improve and update existing systematic information. This basic taxonomic rule is often taken for granted as a central duty (Linnaeus 1751, Fig. 1). Flora texts can provide much information on plant biodiversity (Colasante & al. 2002). With respect to the Italian flora, Pignatti's Flora d'Italia (2017) provides an improved organization; for some critical genera such the genus *Iris* Tourn. ex L., it supplies polytomous diagnostic keys. Such information is essential as a starting point for investigation; it encompasses observation, identification through analytical keys (and their validity), recognition of hybrids as "spies" of active speciation through hybridization, separation between species and hybrids and investigation of their naturalization to better update current data on the qualification and quantification of biodiversity (Colasante & Vosa 1987, 1994, 1995; Colasante & Sauer 1993; Arnold 1997; Colasante & al. 2003; Colasante & Trombini 2003a, 2003b; Colasante 2003a, 2003b, 2004a).



The Beginner
Make all the parts of the Plant more than known
By herbalizing learn to recognize the most common plants by appearance
Collect himself, Dried and accumulate the greatest plants, as many as he can
Learn to distinguish the main parts of fruiting
Familiarize yourself with the Classes and Orders of the system and do not lead back to them the simplest and most obvious flowers
Frequently attend demonstrations in the Botanical Garden
Be clear about the meanings of the Technical Terms, according to their definitions
Examine the Genera known to him, Generic Characters and amend them according to the Genera of the plants, comparing the fruiting with the plants
Draw up descriptions of the species in a workmanlike manner, starting with the simplest plants and continuing with the most difficult.
These discriminations are corrected by the Professor
Himself investigates the species of plants that are unknown to him, according to the Keys, Characters, and Differences of the system
Have a right understanding of the Principles and Fundamentals of Botany
Familiarize himself with the literary history of botany, the authors who have written about plant species should be consulted first.
Get used to scrolling through the Synonyms of the Authors going back to the inventors
Add to the species of the plants the Medical and Economic Use.

The Botanist
The BOTANICAL CHARACTER must be present to the Beginner in a very clear way, so that he can distinguish them Authors from the Scholar Compilers
Cultivate the TRUE BOTANIC botany science everywhere, the Crude Boaster does not make any contribution to the development of science
The true botanist arranges his plants systematically; he does not enumerate them without an order
He recognizes the principle of Fruiting in the theoretical arrangement, he does not change the arrangement according to the Grass
He assumes natural genera; he does not form wrong genera on the basis of the aberrant character of the species
He treats the Species distinctly; he does not create false ones starting from the Varieties
He reduces varieties to species, without equating them to species
He searches and chooses the best Synonyms; it does not settle down, contenting itself with any, obvious nomenclature
He researches the characteristic Differences; he does not prepend specific empty names to real ones
He tries to reduce indefinite Species to Genera; he does not glance at the rarest plants as if they were obvious
He summarizes Descriptions including essential differences, he does not go trumpeting the very natural structure with oratorial speeches
He scrutinizes the Minimal parts carefully; he does not neglect what they effectively explain
Always he illustrates plants with observations; he is not satisfied with a vague name
He observes the singular characters with his own eyes; he does not take his information only from the Authors

THE MASTERS of this Science ascended to the temple of Flora from this ladder.

C. Linnaeus 1751- Botanical Philosophy

Fig. 1. The approach for beginners and botanists to study plants by C. Linnaeus (Botanical Philosophy 1751).

In the context of biodiversity investigations of *Iris* in Italy, we elaborate and correlate a range of appropriate datasets, including chemotaxonomy and cytotaxonomy. We report some new data of particular importance, such as the macro-and micromorphology of *I. statellae* Tod., which was previously little-investigated. We also highlight the conservation *in* ed *ex-situ* of *Iris revoluta* Colas. (Medagli & al. 2014), which has a restricted area distribution, and across-breeding experiment currently underway in Sardinia between individuals of two different populations. These types of synergistic investigations are fundamental to laying the basis for knowledge of the genus *Iris*, in which there is considerable variability of characters both within and between species and populations. Over 300 species of *Iris* are currently reported, all more or less complicated by the numerous natural and artificial hybrids (at least 17000 known, Fig. 7), such as that obtained in our cross-breeding experiment. Since ancient times, irises have been attractive to people for various purposes, including commercial, artistic, symbolic and landscaping (Fusi & al. 1977; Parrini 2006; Fang & al. 2008; Signorini & al. 2009; Tarquini & Colasante 2009a). With their amazing beauty, irises involve humans as either unsuspecting or knowledge able pollinators that contribute to the conservation and expansion of their gene-pool. Moreover, deliberately dispersing their reproductive organs (both vegetative and sexual) has often led to their naturalization after cultivation. This synanthropy has existed for millennia, as graphically reported in world literature and art in China, India, Iran, Mesopotamia, Israel, Turkey, Egypt, Europe and America.

Materials and Methods

For many years, *Iris* populations have been analyzed in the field, distinguishing species with or without hybrids (e. g. in Italian Abruzzo, Apulia, Sardinia, Sicily) and more than 8000 herbarium samples, mainly from Europe and China (Colasante 1983, 1986a; Colasante & Vosa 1986, 1987; Colasante & Sauer 1986, 1988; Colasante & Mathew 1987). Classical investigation techniques have been applied case by case, according to the material, using both macro-and micromorphology (Ross 1892; Dykes 1913; Rodionenko 1961 [ed. 1987]; Wu & Cutler 1985; Köhlein 1987; Blackmore & Barnes 1981), chemotaxonomy (Wynne & Henderson 1973; Cerfon 1981; Williams & al. 1997, 2000 (reprint 2001), karyology and cytogenetics (Bini Maleci 1976; Bini Maleci & Maugini 1981; Brullo & al. 1997; Colasante & Vosa 1981, 1994; Simonet 1932, 1947, 1951; Mitra 1956; Randolph 1957; Randolph & Mitra 1959; Ricci 1958, 1966; Rodionenko 1962; Ricci & Colasante 1974; Ponsaerts 1978). In this paper, we supply some results to highlight the biodiversity of the genus *Iris*, both quantitatively and qualitatively, while focusing on the Italian flora (Table 1).

For some populations, we collected live samples with underground organs (rhizomes, bulbs or stolons, etc.) for the conservation of germplasm in the Botanical Gardens of Rome, providing them with identification labels. We also prepared herbarium samples according to traditional dehydration methods. Both *in-situ* populations and material collected for long-term preservation provided the basis for macro-and micromorphological investigations, the latter focusing on pollen grains, karyotypes and chemotaxonomic analyses using current techniques (Colasante, & al. 1989; Williams & al. 1997, 2000 (reprint 2001); Colasante 2017a). For pollen grain observations, we used a Scanning Electron Microscope (SEM) after dehydration in a critical-point drier (Lynch & Webster 1975; Colasante & al. 1989) and a water-glycerin hanging-drop method for observations made under the light microscope.

Table 1. Material under investigation.

Species	**(rigin**	**Figure**
Species grown in cultivation:		
I. tuberosa (L.) Mill.	Marino, Rome	Fig. 2
I. xiphium L.	Gaeta, Latium	Fig. 2
I. lactea Pall.	Monogeneric Giardino dell'Iris, Florence	
I. triflora Balb.	Botanic Garden and Herbarium, Turin	
I. ensata Thunb.	Monogeneric Giardino dell'Iris, Florence	
I. pseudacorus L.	Riserva Naturale Valle dell'Aniene, Rome; Botanic Garden, Rome	Fig. 2
I. tectorum Maxim.	Botanic Garden, Rome	Fig. 2
I. pseudopumila Tineo	Sannicandro Garganico e Gravine di Laterza, Apulia; Piano Battaglia, Madonie, Sicily	Fig. 4
I. pallida Lam.	Impruneta, Chianti, Tuscany; monogeneric Giardino dell'Iris, Florence, Tuscany; Via Nomentana and Via delle Vigne Nuove, Rome	Fig. 15
I. cengialti Ambrosi A. Kern.	Monte Cengialto, Rovereto	
I. cengialti ssp. *illyrica* (Tomm.)	Poldini and Colasante: Monte Nanos, Slovenia	
Species of natural hybrid origin:		
I. bicapitata Colas.	Gargano	Fig. 4
I. lutescens Lam.	Civitavecchia, Torre d'Orlando; Canino, Viterbo, Latium; Monte Calvi, Monte Pescali, Tuscany	Fig .4
I. marsica I. Ricci & Colas.	Monte Pagano, Val di Rose, more than 10 other populations in National Park of Abruzzo, Latium, Molise	Figs 3, 5
I. relicta Colas.	Monte delle Fate, Latium	Fig. 5
I. sabina N. Terracc.	Monte Gennaro-Zappi, Latium Lazio	Figs 3, 5
I. revoluta Colas.	Scoglio Mojuso, Taranto, Apulia	
I. setina Colas.	Sezze and Monte Pilorci, Latium	Fig. 3
I. sicula Tod.	Monte Busambra and Botanical Garden of Catania, Sicily, Sinis Peninsula and the middle Tirso River valley, Sardinia	Fig. 2
Hybrids of dubious origin and / or escaped from cultivation:		
Iris × sambucina L.	Ponte di Nona and Tor Tre Teste, Rome	
I. × germanica L.	several sites in Latium, Abruzzo, Veneto, Sardinia	Fig. 3
I. × albicans Lange	Vibo Valentia, Calabria; some small populations in Nomentana street, Rome; several populations in Sardinia still under investigation	Fig. 3
I.× florentina L.	Monogeneric Giardino dell'Iris, Florence; Botanical Garden of Perugia; some populations in Latium and Tuscany	Fig. 3

In Sardinia, ongoing population-level studies on *Iris* commenced in 2015 with the subgenus *Iris* section *Iris*. For each site, edaphic parameters, geographic coordinates and samples were collected for further study. Some of these samples were donated to the Botanical Garden of Rome and used for a cross-breeding program carried out in Sardinia using a direct crossing method commonly used by *Iris* hybridizers (Sani & Goretti Specht 1960). To facilitate hybridization, we selected irises with similar macromorphological and phenological characteristics; one of these grows on Carloforte Island (south-western Sardinia) for the female line, and the other in the Sinis Peninsula (central-western coast) for the male line. Pollen from the latter was placed on the lower lip of the stigma of the former to facilitate experimental crossing.

Results

Biodiversity

Biodiversity is generally high, with greater or lesser frequency for the observed species (Colasante 1994, 1995a, b, 2003a, 2004a, 2008, 2013, 2014, 2017a; Colasante & Rudall 2000; Colasante & Pacini 2006; Goldblatt 2000, 2008; Colasante & al. 2004; Lamote & al. 2002; Tarquini & al. 2021a, b), but less so in the beardless irises than in the bearded irises that bear multicellular hairs on the external tepals (Genus *Iris* subgenus *Iris* section *Iris*: Mathew 1981).

Anthesis

Anthesis can occur in winter, spring or summer, according to the different species. In section *Iris*, anthesis occurs mainly in early or late spring, commonly with ephemeral flowers (Colasante & Sauer 1993, Colasante 2004b, 2013, 2014, 2017b).

Habitat

Collectively, habitats are very variable, represented by shallow lakes and marshes (*I. pseudacorus* L., Fig. 2), dry margins of streams and lakes (*I. lactea* Pall.), meadows and olive groves (*I. tuberosa* (L.) Mill., Fig. 2), various substrates – including roofs – in Japan (*I. tectorum* Maxim., Fig. 2), rocky drained places variable in altitudes according to species, from sea level up to over 1000 meters (e.g. *I. bicapitata* Colas., *I. lutescens* Lam., *I. setina* Colas., *I. sabina* N. Terracc., *I. relicta* Colas., *I. marsica* I.Ricci & Colas.), rocky and dry places in Sicily and Sardinia (*I. sicula* Tod. Fig. 2) and marine islets (*I. revoluta* Colas., Fig. 8).

Identification, Characters and Polymorphism

Species identification is complex (Fig. 3) due to high variability of characters observed at all levels: macro-and micromorphological, cytogenetic (Colasante & Vosa 1981, 1995; Colasante & Pacini 2006; Colasante & al. 2003, Colasante & Trombini 2003a, b, 2005a), anatomical (Ross 1892; Rudall, 1994), histological, chemical, chemotaxonomic, biomolecular (Colasante & De Dominicis 1989; Wilson 2011). Pollen grains analyzed by SEM and light microscope (such as the experimental ones of *I.* sp. from Sardinia: Fig. 6) gave good results. Pollen grains show a wide range of patterns in sexine organization and ornamentation, and provide important information for variability as well as for hybrid study and their origin and phylogeny (Arnold & al. 1991; Colasante 1998a; Colasante & Rudall 2000–2001).

Populations of *I. tuberosa* (L.) Mill. (Fig. 2) in Italy show inter- and intrapopulation flower and leaf polymorphisms (Tarquini & al. 2021), as do irises of subgenus *Iris* section *Iris* such as *I. pseudopumila* Tineo (Colasante & Ricci 1979; Colasante 2008; Fig. 4), *I. lutescens* Lam. (Colasante 1986b, 2014; Fig. 4), *I. bicapitata* Colas. (Colasante 1989; Colasante & Trombini 2005a, b; Fig. 4), *I. marsica* I. Ricci & Colas. (Colasante & Ricci 1977; Fig. 3), *I. pallida* Lam. subsp. *pallida* and subsp. *cengialti* (Ambrosi ex A. Kern) Foster (Colasante 2014).

Leaf shape varies at the interspecific level in the traditional subgenera and sections (Ross 1892; Wu & Cutler 1985), but it is quite similar at the interspecific level within the same section (Colasante 1992), except in hybrids. Modified leaves such as bracts are often diagnostic characters at the species level. Flowering stem height is very variable, providing little species diagnostic significance.

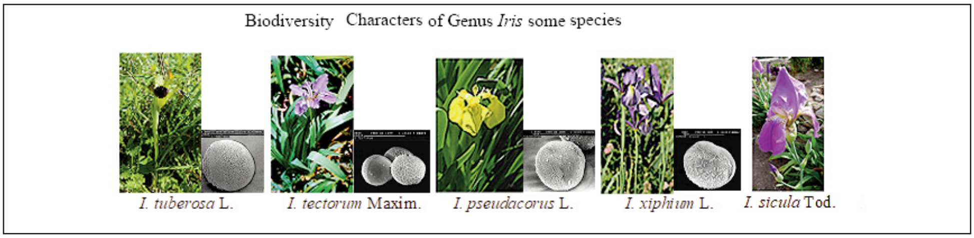


Fig. 2. Biodiversity in the genus *Iris* Tourn. ex L.: species and pollen grain ornamentation.

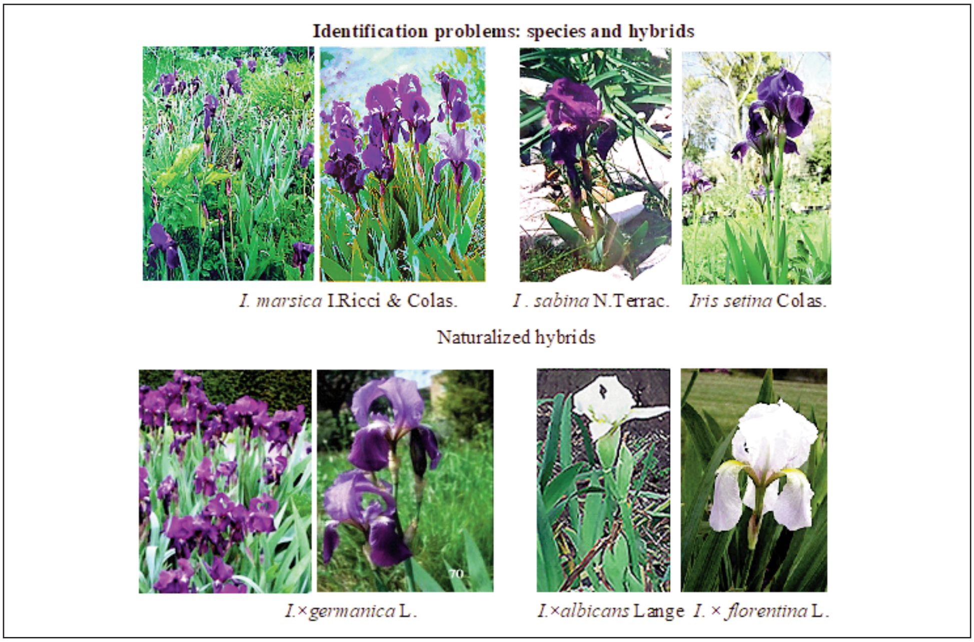


Fig. 3. Identification and problems: species and hybrids. Interspecific variability hinders the real biodiversity in the genus *Iris* Tourn. ex L. subg. *Iris* sect. *Iris* (Mathew 1981).

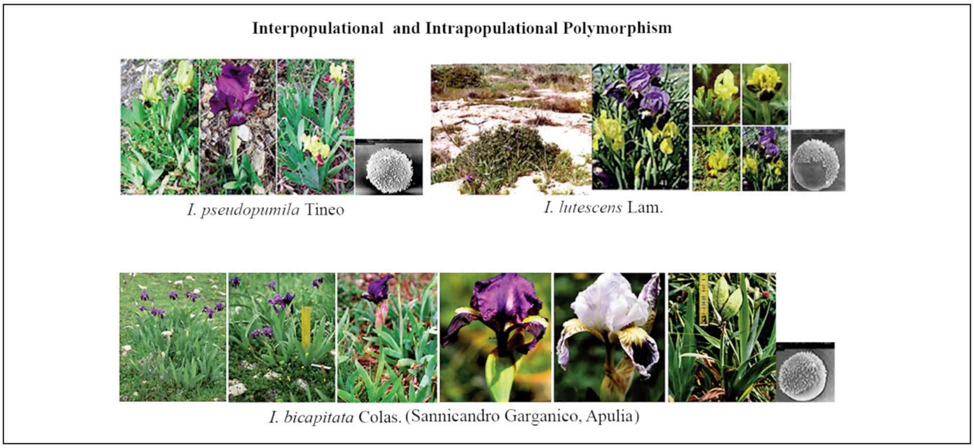


Fig. 4. Inter-and intra- populational polymorphism in the wild populations of *Iris pseudopumila* Tineo in Sicily and Apulia, *I. lutescens* Lam. in Latium, and *I. bicapitata* Colas. in Apulia. For each species Pollen grains in SEM are shown on the right.

Polyploidy, species, natural and experimental hybrids

Polyploidy is frequent in subgenus *Iris* section *Iris*; we analyzed karyotypes to identify the presence of some auto- or allopolyploids and their probable origin (Colasante & Rudall 2000–2001; 2013, 2014, 2017a) and consequent phylogenetic correlations (Colasante 1988; Colasante 2000a, 2000b, 2003a, 2003b, 2004b; Colasante & al. 2003; Goldblatt 2000, 2008; Tarquini & Colasante 2009b). Furthermore, we compared the habits of the species, their pollen morphology (Fig.15), karyotype idiograms of some species at $2n = 40$, with the pair of chromosomes indicative of their diversity (Fig. 5).

Among the bearded irises analyzed, some clearly show natural hybrid origin and good fertility (e.g. *I. bicapitata*) whereas others show lower fertility, albeit caught in the process of stabilization and tendency to introgression with at least one of the progenitors (Colasante 2003a, b; Colasante & Trombini 2003a, 2003b; Colasante & al. 2003). Irises collected in Sardinia used for cross-breeding experiments often produce fewer capsules in nature. Upon initial examination, both sets of taxa appear to be of possible hybrid origin. A high rate of sterility became evident, which is also suggested by the presence of numerous malformed and/or abortive pollen grains (Fig. 6). However, a certain degree of fertility was preserved, as confirmed by experiments reported here. A 5.5 cm capsule containing 16 slightly pear-shaped seeds was obtained (Fig. 6). All seeds were healthy and viable and sprouted in the first year in common-garden substrate. The seedlings produced the first flowers in the second year, with one or two flowers at the stem tip. The specimens born from this crossing are still juvenile because maturity is typically reached from 3 to 5 years of age. The general morphology is largely preserved in the hybrids, but the height is smaller (21.5 cm) than that of the parents measured for three years (54.9 ± 7.5 cm and 56.5 ± 3.5), as we expected given the young age of the seedlings (Simonet 1951, 1955, 1962; Werkmeister 1981; Arnold & al 1991; Hodges & al. 1996; Colasante 2004; Bianconi & al. 2006; Tarquini & Colasante 2009b, 2010a;).

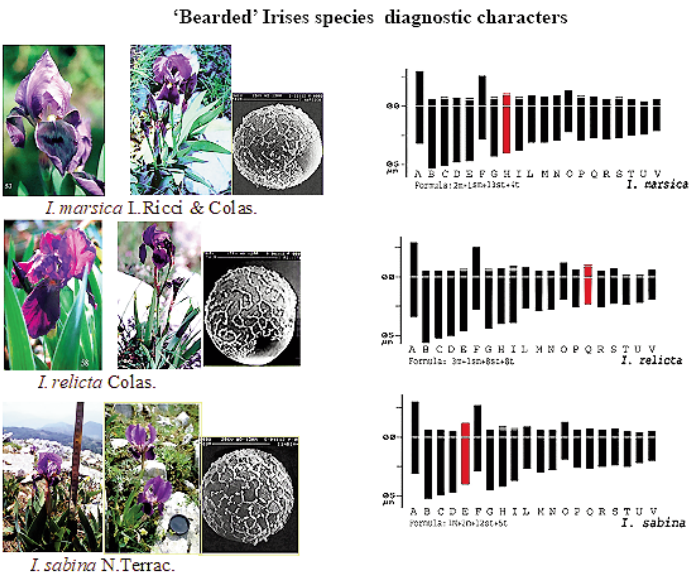


Fig. 5. Species, SEM pollen grain ornamentation and chromosome idiograms of three Italian species with 2*n* = 40 chromosomes. The chromosome pair identifying each species is outlined in red.

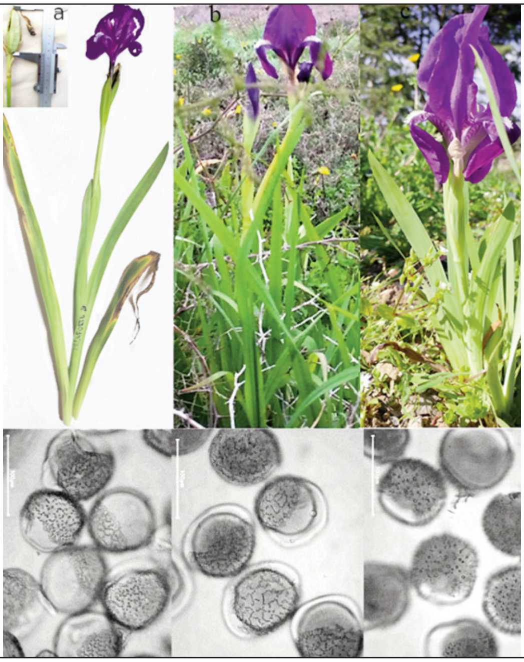


Fig. 6. Iris experimental hybridization: plants and LM pollen grains of the taxa undergoingcross-breeding experiments in Sardinia. (a) Female parent *I. sp.* from Carloforte Island and its capsule; (b) male parent *I. sp.* from the Sinis Peninsula; (c) one of the F1 hybrids obtained.

Molecular and Taxonomic problems

Future studies will require a more targeted phylogenetic approach to help resolve the existing classification of *Iris*. Currently the traditional subgenera and sections (e.g. Mathew 1981) are seriously undermined by the well-supported plastid phylogenies of Wilson (2011) and Wilson & al. (2016), so that the current data appear questionable (see also Colasante & De Dominicis 1989; Reeves & al. 2000; Rycheva & al. 2011).

Chemotaxonomic investigations can provide useful specific taxonomic and nomenclatural markers. Some compounds are common to several species, others are present in only a single species, such as apigenin 7,4'-dimethyl ether-6-*C*-glucoside 2"-O-rhamnoside in *I. pallida.* This technique can offer very detailed information and allow separation even between species and hybrids (Williams & al. 1997, 2000–2001). From the taxonomic viewpoint, the relatively low knowledge of *I. sicula* Tod. (1858) (Fig. 2) was evaluated in relation to the other taxa considered similar, such as *I.* × *mesopotamica* Dykes (1913), *I.* × *germanica* L. (1753) (Fig. 3), *I.*× *trojana* A. Kern. ex Stapf. (1887) and *I.* × *cypriana* Foster & Baker (1888).

Many of these taxonomic problems remain the subjects of ongoing studies, one of which concerns the inaccurate identification of *I. sicula* and its erroneous attribution to either *I. pallida* or *I.* × *germanica* according to different authors (see Discussion).

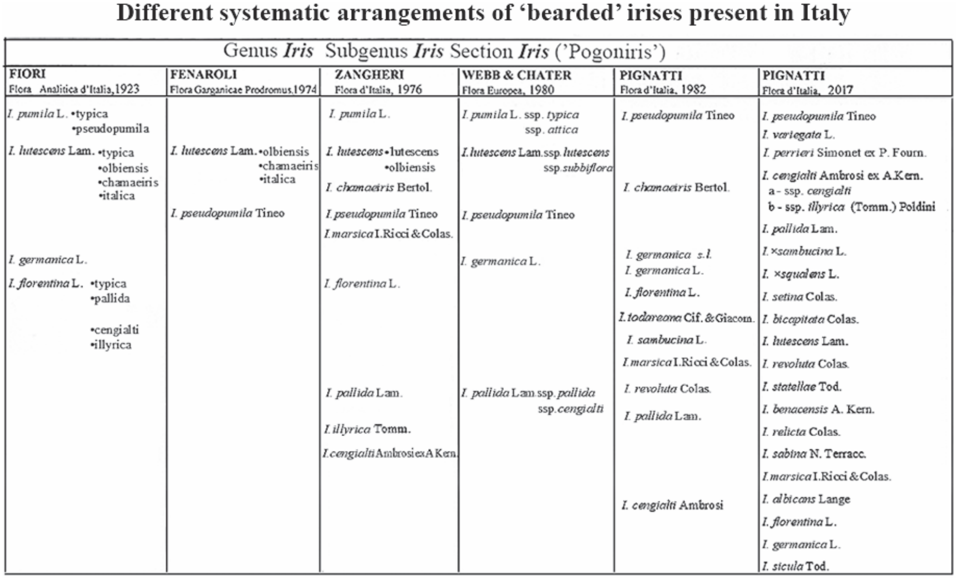
Dissemination of scientific data

Screening of scientific data (e. g. from populations, Herbaria, Botanical Gardens, scientific literature, Flora accounts and dedicated websites) highlights much incorrect or misleading information due to the inaccurate, superficial or erroneous interpretations of the initial sources, as happened for *I. mesopotamica* (see Discussion). Actually, all this affects the exact knowledge and dissemination of real biodiversity. Even the exchange of labels between Herbaria and Botanical Gardens can lead to incorrect attribution. This type of confusion in identification has unfortunately resulted inconsiderable proliferation of species (Parlatore 1858; Terracciano 1899; Lynch 1904; Dykes 1913; Fiori 1923–1929; Lawrence 1953; Werkmeister 1967; Zodda 1967; Fenaroli 1974; Zangheri 1976; Haslam & al. 1977; Warburton 1978; Pignatti 1982, 2017; Innes 1985; Festi 1986; Bianco 1991; Waddick & Zhao 1992; Terpenin & al. 1996; British Iris Society 1997; Poldini 2001; Poldini & al. 2002; Pries 2006; Conti & al. 2005a, 2005b, 2007; Giardina & al. 2007; Camoletto 2008; Camoletto & al. 2009; Arrigoni 2010; Giardina 2010; Mathew 1981; Minà & Palumbo 2011; Mitić & al. 2013).

In the Botanical Garden of Rome (Figs. 11, 12, 13), some *Iris* species have been collected from their *locus classicus* to form systematic *ex-situ* collections placed more or less according to their preferred habitat in systematic-ecological plant beds, with the goal to safeguard the conservation of their genic pool (Figs. 7, 11-13). About thirty years ago, the Italian Iris Society of Florence donated rhizomes of experimental hybrids of subgenus and section *Iris* in a twinning initiative with the monogeneric Garden of Iris (Fig. 14), which hosts hybrids for an international iris competition that has been held every year for more than fifty years (Maugini 1992; Bianconi & al. 2006). This initiative also involves many other iris societies worldwide. Other institutions have also been involved in developing the iris collection, such as San Galliano in the Marche, the Rea Botanic Garden in Trana, Turin, and the University of Perugia. After years of rhizome exchange, there are currently beautiful collections of iris varieties in the Iris Garden of Florence and in the Orto Botanico di Roma, mainly of bearded irises used in the spring for educational exhibitions and cultural seminars for universities, institutes, schools and tourists.

Furthermore, as evidence of their progress over the years, some individual Italian hybridizers have donated experimental hybrids, including Augusto Bianco (Piedmont), Tiziano Dotto (Veneto), Amedeo Fadda (Sardinia) and Giovanni Edoardo Nogaro (Lombardy). Thus, the *Iris* germplasm bank has expanded considerably through the last fifty years in Italy (Table 2) and is now very rich in species, subspecies, hybrids and varieties (Fig. 7).

Table 2. Systematic arrangements of bearded irises present in Italy in six Flora texts.



Different systematic arrangements of 'bearded' irises present in Italy

Genus ***Iris*** Subgenus ***Iris*** Section ***Iris*** ('Pogoniris')

FIORI Flora Analitica d'Italia,1923	**FENAROLI** Flora Garganicae Prodromus,1974	**ZANGHERI** Flora d'Italia, 1976	**WEBB & CHATER** Flora Europea, 1980	**PIGNATTI** Flora d'Italia, 1982	**PIGNATTI** Flora d'Italia, 2017
I. pumila L. •typica •pseudopumila		*I. pumila* L.	*I. pumila* L. ssp. *typica* ssp. *attica*	*I. pseudopumila* Tineo	*I. pseudopumila* Tineo *I. variegata* L.
I. lutescens Lam. •typica •olbiensis •chamaeiris •italica	*I. lutescens* Lam. •olbiensis •chamaeiris •italica	*I. lutescens* •lutescens •olbiensis	*I. lutescens* Lam. ssp. *lutescens* ssp. *subbiflora*		*I. perrieri* Simonet ex P. Fourn.
		I. chamaeiris Bertol.		*I. chamaeiris* Bertol.	*I. cengialti* Ambrosi ex A.Kern. a - ssp. *cengialti* b - ssp. *illyrica* (Tomm.) Poldini
	I. pseudopumila Tineo	*I. pseudopumila* Tineo *I. marsica* I.Ricci & Colas.	*I. pseudopumila* Tineo		*I. pallida* Lam.
I. germanica L.			*I. germanica* L.	*I. germanica s.l.* *I. germanica* L.	*I. ×sambucina* L. *I. ×squalens* L.
I. florentina L. •typica •pallida •cengialti •illyrica		*I. florentina* L.		*I. florentina* L. *I. todareana* Cif. & Giacom. *I. sambucina* L. *I. marsica* I.Ricci & Colas.	*I. setina* Colas. *I. bicapitata* Colas. *I. lutescens* Lam. *I. revoluta* Colas.
		I. pallida Lam.	*I. pallida* Lam. ssp. *pallida* ssp. *cengialti*	*I. revoluta* Colas. *I. pallida* Lam.	*I. statellae* Tod. *I. benacensis* A. Kern.
		I. illyrica Tomm. *I. cengialti* Ambrosi ex A Kern.			*I. relicta* Colas. *I. sabina* N. Terracc.
				I. cengialti Ambrosi	*I. marsica* I.Ricci & Colas. *I. albicans* Lange *I. florentina* L. *I. germanica* L. *I. sicula* Tod.

Fig. 7. Iris cultivars: colors and forms in several modern artificial hybrids highlighting the variability increased by cross-breeding activities.

Distribution

Generally, the distribution of the species does not correspond to the real one due to misidentification of the iris in question which is then erroneously represented also in maps. New researches were carried out in the field, in herbaria and Flora texts to reduce the misinformation about identifications. For the Italian subgen. sect. *Iris* a process of updating of the map Region by Region is in progress (Colasante 1987, 2014; Colasante & Altamura 1986, 1988; Colasante & Mathew 1987 – Fig. 10). Remaining problems need to be solved species by species for the taxonomic reasons outlined above, including abundant initial misidentifications. For Sardinia, investigations are underway with the aim of confirming and updating the information necessary for proper identification and consistency of the iris populations. The first results confirm the presence of *Iris foetidissima* L., *I. pseudacorus* L., *I. planifolia* (Mill.) Fiori & Paoletti, *I.* × *germanica*, *I.* × *albicans* Lange and *I. sicula*, the latter erroneously synonymized with *I. pallida* Lam. subsp. *pallida* (Colasante 2017a). Therefore, some stations reported for *I. pallida* subsp. *pallida* (Arrigoni, 2010) host *I. sicula,* whereas *I. pallida* itself appears confined to gardens and crops. Other irises of subgenus *Iris* section *Iris* remain in need of further investigation (Fadda & al. 2021).

Discussion

A primary goal of biosystematic research in plants is to create natural classifications, which are often complicated by convergence and hybridization (Arnold 1997; Arnold & al. 1991; Cruzan & Arnold 1993, 1994; Hodges & al. 1996; Colasante 1998a, 2000c, 2003b, 2004a, 2004b; Colasante & Pacini 2006; Colasante & Trombini 2006a, 2006b; Colasante & Tarquini 2006; Colasante & Mathew 2008). A systematic approach for irises has highlighted the problem of their high level of biodiversity, influenced strongly by speciation through allopolyploidy (Colasante 1988, 1992, 1996a), hybridization, cultivar naturalization and their distribution by man. Even today we can readily find highly critical areas of research to be highlighted and discussed, demonstrating the need for synergy in scientific data to facilitate more precise identification of species, hybrids and garden varieties.

The high level of biodiversity in the results could result partly from an easy environmental-ecological adaptation of taxa and their genetic richness. The relatively short life or ephemeral nature of many iris flowers can represent a barrier to hybridization, though it can occasionally facilitate it (Colasante & Rudall 2000-2001). However, flowering times are variable throughout groups of species of the same subgenus and section, and contemporary flowering can occur even in the same section, due to different causes. Climatic variation, natural and anthropogenic barriers and habitat destruction can all lead to hybridization by anticipating or postponing the flowering period (Fig. 15) and preventing genetic erosion (Colasante & Sauer 1993).

Identification, Characters and Polymorphism

The initial approach to understanding an iris species or taxon requires observation of a wide range of possible characters (Linnaeus 1751, Figs. 1, 15). If the taxon shows intermediate characters or variability in the same population, morphology alone is rarely sufficient to immediately identify a new species or subspecies and further investigation is needed (Colasante & Vosa 1987; Colasante 1992, 1997, 1998a, 1998b; 2004b, Colasante & Trombini 2005a; Colasante & Mathew 2008). A fundamental prerequisite in providing new reliable information on plant biodiversity involves the comparison of different information concerning the origin of the taxon, observation of the population in the *locus classicus*, variability, hybrids and their probable ancestors, related species and the existence of naturalized cultivars. A single character can lead to misleading results that can negatively affect perceived nomenclature, taxonomy and species distribution.

In a highly specialized genus such as *Iris*, exhibiting rich population polymorphism, it is relatively easy to find misidentified taxa, even sometimes confusing ancestors with descendants. Even the leaves can show variability in the different subgenera and sections of the genus (Ross 1892; Wu & Cutler 1985), though leaf anatomical characters in hybrids can indicate their origin and progenitors (Colasante 2004b). In the flowers, morphometric studies show that the length of the perigonium tube is fairly indicative of species, but not always so in natural and experimental hybrids because it can have a variable length range between those of progenitor species or even coincide with one of them. Such polymorphism is therefore not readily informative for taxon separation (Colasante 1995a, b; Colasante 1998a). Other characters can also vary, such as the stem length, which is influenced by plant age, edaphic conditions and altitudinal position. Variation in stem height has been used for classification purposes (Lawrence 1953) but is misleading because it is rarely constant in nature, often varying in the same population and sometimes in the same individual in different years (e. g. *I. pseudopumila*, *I. lutescens*, *I. bicapitata*; Fig. 4). Flowering stem length in the subgenus *Iris* section *Iris* can be considered only for hybrid varieties. Hence, polymorphism is common within and between species and populations in terms of shape, flower colour and distribution. Polymorphism greatly misleads correct identification of species and leads to their proliferation (e. g. *I. lutescens* and *I. chamaeiris* Bertol.). Over-estimation of species numbers and under-estimation of their polymorphism together create many taxonomic synonyms. In other irises, errors of identification can result from superficial choice of diagnostic characters (e. g. *I. benacensis* A. Kern. ex Stapf. and *I. aphylla* L.: Colasante & Mathew 2008, Colasante 2014, 2017a)

Polyploidy and Hybridization

Polyploidy is frequent in natural populations, often with bimodal karyotypes, either as auto- or allopolyploidy; both are used by the progenitor species of natural hybrids to stabilize or defend themselves from environmental stresses. Allopolyploid evolutionary processes against extinction are evident in the crossing between *I. pseudopumila* and *I. reichenbachii* Heuff. to get to *I. sicula,* perhaps following a genome-doubling event (Colasante & al., 2003a; Tarquini & Colasante 2009b; Colasante 2017a). One possible accidental outcome of allopolyploidy could be to help preserve the gene pool from erosion. Allopolyploidy is also useful for experimentally obtaining new hybrids and varieties via new crosses (Foster 1889; Mahan 2007). This latter approach can be achieved taking into account the cognitive background and degree of observation of the researcher and hybridizer, and also the conditions of the specimens, age and size of the plant, timing of anthesis and their sexual and vegetative reproduction, presence of polymorphism, hybridization even in nature, as ascertained by the cytogenetic studies reported here (Figs. 4, 5). In the new experimental hybrid between Sardinian taxa, the progenitors show some common characteristics in anthesis time, macromorphology and phenology, and strengthen the supposition to have at least one common ancestor (all ongoing studies and yet to be fully demonstrated). This result suggests a probable introgressive cross that reinforces the "dominant" progenitor characters.

Molecular and Taxonomic characters

To date, no single biomolecular technique is sufficient to detect 'bearded' iris hybrids and separate them from their progenitors, whether species or hybrids (Colasante & De Dominicis 1989; Colasante 2000d; Colasante & Vosa 2000; Reeves & al. 2000; Lamote & al. 2002; Wilson 2011; Raycheva 2011), though in future a combination of next-generation sequencing will help solve many remaining problems.

In our studies, a chemotaxonomic compound (Luteolin di-C-glycoside) strongly characterizes *I. reichenbachii* and is absent from the other species of the same section examined by us (Williams & al. 1997, 2000–2001; Harborne & Williams 2001). Similarly, *I. pallida* is strongly characterized by apigenin 7,4'-dimethyl ether-6-*C*-glucoside 2''-O-rhamnoside. This technique offers detailed information that allows separation between species and hybrids and positively supports cytotaxonomic data of autopolyploidy and allopolyploidy.

Threatened, rare and/or endangered species, Botanical Gardens and Conservation

Many irises of subgenus *Iris* section *Iris* require protection, some of them urgently. Among possible extinct species, *I. statellae* Tod. was known for Sicily in the last century but has not been found during approximately the past 40 years This species shows partially semitectate pollen grains (Fig. 8), and an allopolyploid karyotype with 2*n* = 40. Moreover,

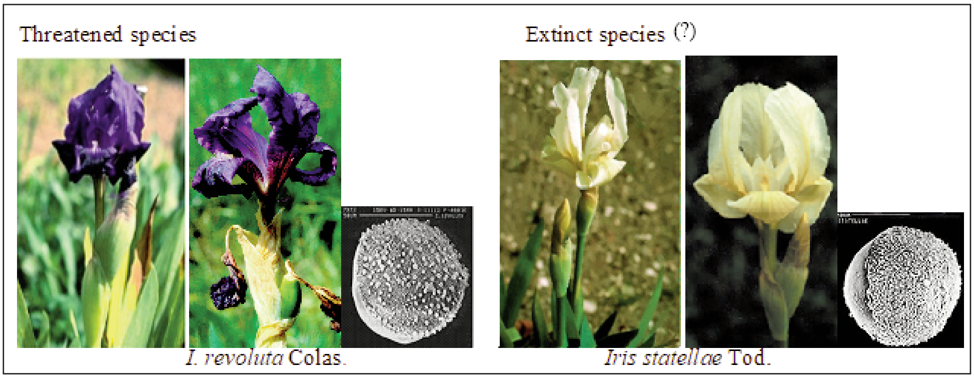


Fig. 8. Conservation: plants and (on right) SEM pollen grains of the Italian threatened species of *I. revoluta* Colas., which is subject to protection measures, and *I. statellae* Tod. (showing pollen grain ornamentation and chromosome number 2*n* = 40), which is presumed extinct.

I. revoluta Colas. (Fig. 8), with a very limited distribution, has been subjected to *ex-situ* and *in-situ* conservation to reduce its threatened range of extinction (Accogli & al. 2008; Medagli & al. 2014). Species and any subspecies to be considered more or less threatened by range or genetic impoverishment include *I.* × *albicans* Lange, *I. benacensis* A. Kern., *I. bicapitata* Colas., *I. cengialti* Ambrosi, *I. foetidissima* L., *I.* × *germanica* L., *I. graminea* L., and its variety 'pseudocyperus' Schur., *I. juncea* Poir., *I. lutescens* Lam., *I. marsica* I.Ricci& Colas., *I. pallida* Lam., *I. perrieri* Simonet ex N. Service, *I. planifolia* (Mill.) Fiori and Paoletti, *I. pseudacorus* L., *I. pseudopumila*Tineo, *I. relicta* Colas., *I. sabina* N.Terracc., *I.* × *sambucina* L., *I. setina* Colas., *I. sibirica* L., *I. sicula* Tod., *I. sintenisii* Janka, *I.* × *squalens* L., *I. tuberosa* (L.) Mill., *I. unguicularis* Poir., *I. variegata* L. and *I. xiphium* L. (Colasante & Rudall 2000). *Iris sicula* must be placed in the species protection list also for Italy; in Malta it is already protected by law (Schedule III and VI of legal notice LN311 / 2006), as well as in Israel where it is reported as *I. mesopotamica* and included in the IUCN Red List (Sapir & al. 2016, IUCN).

Systematic problems

There are examples of both species- and genus proliferation in *Iris* classification. For genera, we highlight the case of a monospecific taxon that still causes debate on its attribution to either the genus *Iris* (*I. tuberosa*) or *Hermodactylus* (L.) Mill. (Tillie & al. 2000–2001). Some botanists underestimate the difference in the arrangement of the ovary due to the complete or incomplete margin of the septa (Colasante 2014). However, plastid data strongly indicate that *Hermodactylus* belongs within the genus *Iris* (Wilson 2011).

With regard to species proliferation, we identify several demonstrable examples of inaccurate and erroneous synonymization:

Fig. 9. Type specimens of (a) *I. bicapitata* Colas., (b) *I. biflora* L. and (c) *I. biflora sensu* Petagna.

(1) *Iris biflora* L. (1753, Fig. 9b) was so named due to its anthesis observed twice a year as written in the accompanying note. The establishment of a new species with the same binomial (*I. biflora sensu* Petagna, 1787, Fig. 9c) was a misinterpretation by Petagna, who, misled by the presence of two flowers at the flowering stem apex, misunderstood the first author's note about the combination given to this taxon. In fact, *I. biflora sensu* Petagna (1787) from Apulia, is a new species and has now been assigned the binomial *I. bicapitata* Colas. (Colasante, 1996b; Figs. 4, 9a). Despite this clarification, subsequent studies were carried out on *I. biflora* L. with a proposal to reject this new name (Boltenkov & Crespo, 2019).

(2) Another example concerns *I. mesopotamica* Dykes (1913), which many botanists synonymized with *I.* × *germanica* L., but recent studies (Colasante 2017a) have shown is actually synonymous with *I. sicula* Tod., so the *I. mesopotamica* binomial should be rejected.

(3) *Iris sicula* described by Todaro in 1858, at $2n = 48$, was erroneously synonymized by some authors with *I. pallida* Lam., at $2n = 24$ (Tutin & al. 1980; Arrigoni 2010) and by others with *I.* × *germanica* L., at $2n = 44$ in many Flora texts, floristic lists, reports and websites (Norris 2012; Nowick 2015; WCSP 2019). This suggestion would lead also to synonymizing *I. pallida* Lam. with *I.* × *germanica* L., the latter often confused also with *I. marsica* ($2n = 40$), which is completely unacceptable for all of the cytogenetic, morphological and chemotaxonomical characters that distinguish these taxa. Our results also show that in *I.* × *germanica* L., other taxa and in particular *I.* × *trojana* and *I.* × *cypriana* show a similar problematic situation that prevents genuine understanding. All this reasoning leads us to separate *I.* × *trojana* and *I.* × *cypriana* from *I. sicula*.

(4) A critical situation occurs for *I. perrieri* Simonet ex P. Fourn. (1935) in Piedmont, misidentified and considered incorrectly as a taxonomic synonym of *I. aphylla* L. (1753) in reports, Herbaria, floristic lists and Flora texts (Rotti 1992; Colasante & Mathew 2008; Colasante 2017a), as well as for beardless irises such as *I. lactea* Pall. (1776), *I. triflora* Balbis (1804–1806) and *I. ensata* Thunb. (1794) that show numerous taxonomic and nomenclatural synonyms (Colasante 2009). The perpetuation of unreliable information in texts and websites continues to mask the real biodiversity among these irises and highlights how much criticality still may exist within this genus.

Distribution data and synergy

The erroneous or limited knowledge of the distribution of individual taxa, as well as the proliferation and/or lack of separation of the species, is spread chaotically through mass dissemination and websites, Herbaria, Botanical Gardens and Floras. In sympatric populations of species and related natural hybrids, morphological characters alone often do not allow us to confidently distinguish species from hybrids, especially if the hybrids assumed many characters of the dominant progenitor. Any morphological asymmetry can be increased by introgressive hybridization (e. g. sympatric populations as in Sannicandro Garganico, Apulia, where *I. bicapitata* is present with *I. pseudopumila* as one of its ancestors: Williams & al. 2000 - 2001). Therefore, for a "botanist", under-estimating the "minimum parts" (Linnaeus 1751, Fig. 1) is one of the major impediments to correct identification of the sample (individual, population, species, hybrid, variety). The distribution should be summarized at the end of the studies (Fig. 10), after reviewing synergistically data available from other sources (Fig. 15), including morphology and field-based morphometrics as a first step. Only synergistic surveys can lead to real biodiversity data allowing us to make well-informed decision when prioritizing species for protection (Simonet 1951, 1955, 1962; Werkmeister 1981; Arnold & al 1991; Hodges & al. 1996; Colasante 2004; Bianconi & al. 2006; Tarquini & Colasante 2009b, 2010a).

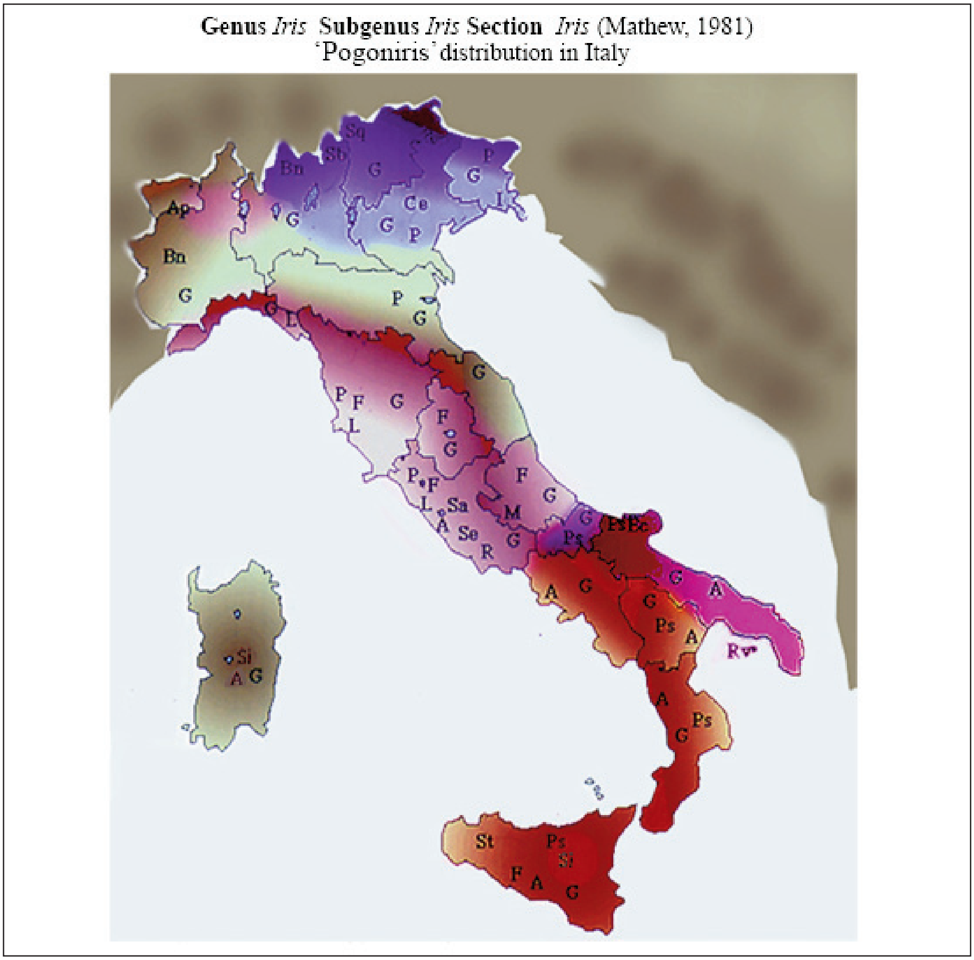


Fig. 10. Updated distribution map of Genus *Iris* Tourn. ex L. subgenus *Iris* section *Iris* (Mathew, 1981). **A** – **I.* × *albicans* Lange , **Bc** – *I. bicapitata* Colas., **Ce** – *I. pallida* subsp. *cengialti* (Ambrosi ex A. Kern.) Foster, **F** – **I.* × *florentina* L., **G** – **I.* × *germanica* L., **I** – *I. pallida* subsp. *illyrica* (Tomm. ex Vis.) K. Richt., Be – *I. benacensis* A. Kern., L – *I. lutescens* Lam., M – *I. marsica* Ricci & Colas., **P** – **I. pallida* Lam., **Pe** – *I. perrieri* Simonet ex N. Service, **Ps** – *I. pseudopumila* Tineo, **R** – *I. relicta* Colas., **Rv** – *I. revoluta* Colas., **Sa** – *I. sabina* N. Terracc., **Sb** – *I.* × *sambucina* L., **Se** – *I. setina* Colas., **Si** – *I. sicula* Tod., **Sq** – *I.*× *squalens* L., **St** – *I. statellae* Tod.
*: cultivated and naturalized in many places of Italy.

Data Dissemination

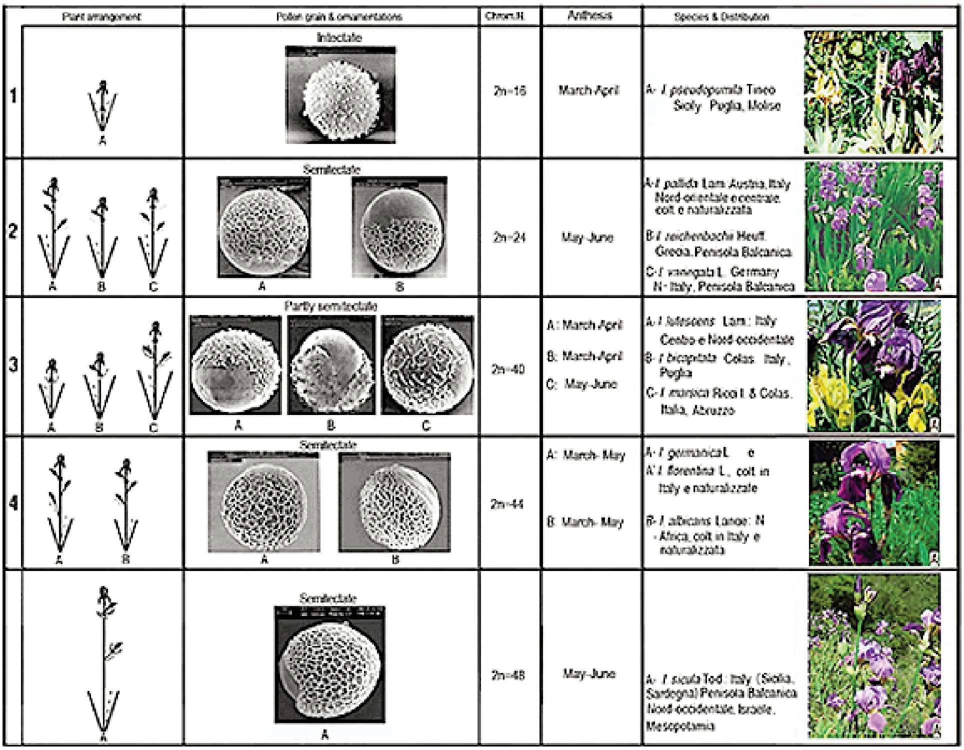
Botanical gardens (Figs. 11-12-13-14) play an important role in this topic (Maunder 2001; Tarquini & Colasante 2010b; Cibrian-Jaramillo & al. 2013); for example, in the dissemination of scientific news, in/ex-situ protection and conservation activities and germplasm collections (Toselli & Bressan 2006; Mounce & al. 2017).

Fig. 11. Rome Botanical Garden: view from above.

Fig. 12. Rome Botanical Garden: Palm path.

Fig. 13. Iris varieties in cultivation.

Fig. 14. The *Iris* monogeneric Garden in Florence at the 53rd *Iris* annual International Competition (2009).



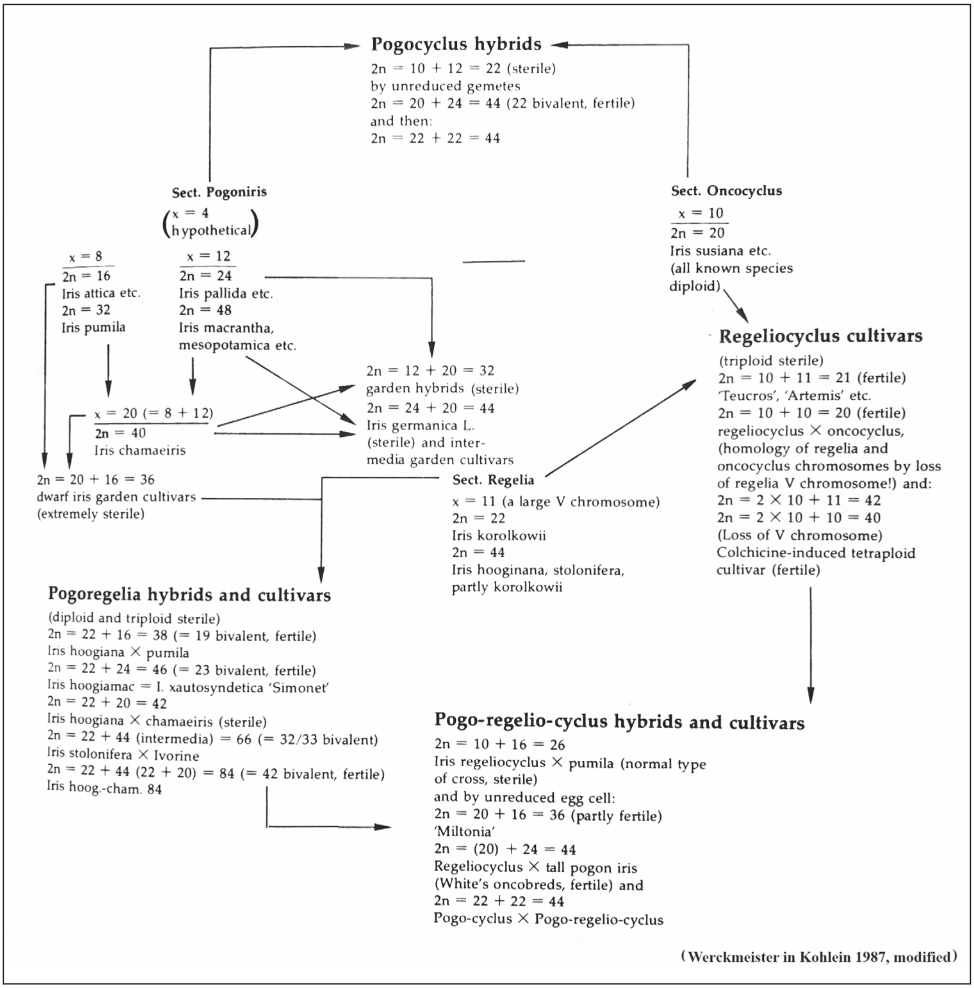
	Plant arrangement	Pollen grain & ornamentations	Chrom.N.	Anthesis	Species & Distribution
1	A	Intectate	2n=16	March-April	A- *I. pseudopumila* Tineo Sicily Puglia, Molise
2	A B C	Semitectate A B	2n=24	May-June	A- *I. pallida* Lam. Austria, Italy Nord-orientale e centrale, colt. e naturalizzata B- *I. reichenbachii* Heuff. Grecia, Penisola Balcanica C- *I. variegata* L. Germany N- Italy, Penisola Balcanica
3	A B C	Partly semitectate A B C	2n=40	A: March-April B: March-April C: May-June	A- *I. lutescens* Lam.: Italy Centro e Nord-occidentale B- *I. bicapitata* Colas. Italy, Puglia C- *I. marsica* Ricci I. & Colas. Italia, Abruzzo
4	A B	Semitectate A B	2n=44	A: March- May B: March- May	A- *I. germanica* L. e A'- *I. florentina* L., colt. in Italy e naturalizzate B- *I. albicans* Lange: N-Africa, colt. in Italy e naturalizzata
	A	Semitectate A	2n=48	May-June	A- *I. sicula* Tod.: Italy (Sicilia, Sardegna) Penisola Balcanica Nord-occidentale, Israele, Mesopotamia

Fig. 15. Genus *Iris* Tourn. ex L., subgenus *Iris,* section *Iris* (*sensu* Mathew 1981): comparative scheme using an integrated-synergistic approach concerning plant arrangement, pollen-grain ornamentation, chromosome number, and anthesis.

Precise dissemination of accurate synergistic data (Fig. 15) is an essential requirement for any serious botanist and germplasm collections (Toselli & Bressan 2006; Mounce & al. 2017).

It is also very important to point out the accurate dissemination of data on the polyploidy of the Tall

Bearded Iris(TBI), often classified according to the height of the flower stem (Lawrence, 1953). A common problem is the widespread misconception that these hybrids are all tetraploid and related to *Iris* × *germanica.* The binomial *Iris* × *germanica* L. is used widely by gardeners and in the horticultural market but it includes several hybrids and cultivars, different from the species described by Linnaeus; this leads to great confusion even in the specialist literature (Norris 2012; Nowick 2015; Tsukatani & al. 2002) and on dedicated websites (WCP 2019). The iris hybrids diagram shown in Fig.16 demonstrates how far these views are from reality about the real identity of *Iris* × *germanica* L.; this scheme is useful to clarify ideas not only to researchers but also to experimental hybridizers and iris lovers (Werckmeister in Kohlein 1981; Colasante 2004b, 2014; Fig.16).



(Werckmeister in Kohlein 1987, modified)

Fig. 16. Diagram of some iris hybrids.

Conclusion

Our synergistic approach will not only avoid superficial conclusions to taxonomic problems and subsequent incorrect dissemination of information but will also emphasize the separation of genuinely distinct taxa. It will help to discourage taxon proliferation and allow the recognition of taxonomic and nomenclatural synonyms, especially regarding highly critical taxa such as the genus *Iris* (notably in *I. lactea* Pall., *I. ensata* Thunb., *Iris sicula* Tod., *I. lutescens* Lam., *I. marsica* I. Ricci & Colas., *Iris × germanica* L. and *I. pallida* Lam.).

Despite our long-term and ongoing studies, this approach is still not universally applied, even to Italian irises, though it remains essential for the conservation and protection of biodiversity (IUCN). We invite experts to adopt adequate measures of investigation across vascular plants, according to the Strategic Plan for the protection of biodiversity, 2011–2020, which remains in force today.

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Note: Actually, many web-sites on irises, but not all providing scientific data and many shows dubious information and even inaccurate (sometimes personally commented out), but according to the purpose of clarifying of this paper only sites of scientific public domain were carefully checked and, in particular, for the update nomenclature, WCSP, Kew (2019), which, if accepted also by these authors, have been followed.

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