



An overview on studies of species complexes in Solanaceae

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Received: February 7, 2023

Accepted: June 6, 2023

ABSTRACT

Solanaceae comprises many species complexes, taxonomically challenging lineages that require specialized effort to be reliably delimited, and thus develop a reasoned hypothesis at the species level. To obtain an overview of aspects that permeate species complexes studies in Solanaceae, we collected and summarized details of selected works, resulting in 83 published articles comprising nine genera. *Solanum*, the most studied genus, spans all explored biogeographic realms, characterized by taxonomic complexity related to its long history of domestication. *Capsicum* is a unique case due to the adoption of complexes as an indicator of gene pool, while *Petunia* can potentially serve as a model for the use of species complexes to improve evolutionary knowledge given their phylogeographic studies. The Neotropical region concentrates the majority of research and presents the highest number of genera studied. Morphometrics is the main applied approach probably due to its low cost, followed by population genetics, reproductive biology, phylogeny, and others. Most studies do not present taxonomic decisions or apply integrated methods. We encourage studies with some neglected genera that may have hidden species complexes; a major effort to resolve the *Solanum nigrum* complex; and the use of effective, less applied fields of study such as ecology and palynology.

Keywords: Biogeographic realm, *Capsicum*, cryptic species, domesticated taxa, morphometrics, *Petunia*, *Solanum*, taxonomy, nightshades.

Introduction

Solanaceae is a family with 96 genera and approximately 2,400 species distributed among all tropical and temperate regions of the world (Barboza *et al.* 2016). Most of its species richness, however, is concentrated in the western hemisphere, particularly in South America, where the family is more diversified and has the largest number of endemic genera (Hunziker 2001; Olmstead 2013; Dupin *et al.* 2017).

Despite being a “medium-sized family” (Solanaceae Source, <https://solanaceaesource.myspecies.info>), Solanaceae stands out among flowering plants for its long history of human domestication (Daunay *et al.* 2007) and economic importance, comprising many crop species such as potato (*Solanum tuberosum* L.), tomato (*Solanum lycopersicum* L.), eggplant (*Solanum melongena* L.), chili peppers (*Capsicum* spp.) and tobacco (*Nicotiana tabacum* L.), as well as ornamental flowers like petunia (*Petunia x hybrida* (Hook.)).

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Vilm.) and trumpet flower (*Brugmansia* spp.) (Barboza *et al.* 2016). Solanaceae is commonly known as the “nightshade family” and is composed primarily of perennial herbaceous and woody plants with such marked morphological diversity that it is difficult to find characteristics universally shared by its members (Hunziker 2001). Still, many species have conspicuous solitary or clustered insect-pollinated flowers; five fused sepals and petals; five stamens; superior ovary composed of two carpels fused and placed obliquely in the flower on a basal disk of tissue; and simple style with a two-lobed stigma (Morris & Taylor 2017). Also, a variety of leaf traits, chromosome number, and indument types are present in the family (Barboza *et al.* 2016). Solanaceae contains a wide variety of secondary metabolites, with at least nine types of alkaloids, as well as over 300 types of withanolides, the most common steroid within the family, of biological and pharmacological importance (Hunziker 2001; Eich 2008). The species of the family are known to be poisonous, especially due to toxic members such as the deadly nightshade (*Atropa belladonna* L.), henbane (*Hyoscyamus niger* L.), mandrake (*Mandragora* spp.) and the carcinogenic *Nicotiana tabacum* (and relatives) (Lee 2006). The latter is probably the plant whose use has led to the highest number of human deaths due to consolidation of tobacco as a global legal drug (Drope *et al.* 2022).

Solanaceae has always received considerable attention from botanists regarding the systematic relations within the family (D’Arcy 1979; 1986; Cronquist 1981; Hunziker 2001). Recent studies have provided a better understanding of the evolutionary relationships between higher clades and established a calibrated phylogeny (Olmstead *et al.* 2008; Särkinen *et al.* 2013). However, many clusters at the terminal nodes of the evolutionary tree are poorly resolved and raise questions about the morphological characteristics that define the delimitation of taxa. Some genera, such as *Solanum* L. and *Capsicum* L., can be particularly challenging to identify and classify because of their natural diversity and similarities between wild and domesticated species (Van den Berg *et al.* 1998; Ince *et al.* 2010). Also, many groups in Solanaceae, especially *Solanum*, represent a “taxonomic paradox”, presenting both strong morphological diversity and uniformity (Roe 1972), leading to the creation of countless names, including infraspecific categories. Many of these groups have received considerable attention over the advancement of studies in Solanaceae and are currently considered species complexes (e.g., Bukenya & Carasco 1994; Van den Berg *et al.* 1998; Zhang *et al.* 2008; Silvar & García-González 2016).

There is a broad understanding of the concept of species complex, still lacking further elucidation by the research community. An interesting example is the question “what is a species complex?” which mobilized high social media engagement on a ResearchGate forum in 2016 (<https://researchgate.net/post/What-is-a-species-complex>; accessed August 2022). When looking at the 51 answers it received, the

complexity of the topic is immediately unveiled. According to the scientists who participated in the forum, a species complex: (i) may represent more than one species, (ii) it does not have well-defined taxonomic boundaries, (iii) involves phylogenetically related taxa, (iv) it can be a collection of species assumed to be populations or subspecies of a single species, (v) it is a result of the inaccuracy of current methods, (vi) it is an even more complicated hypothesis than the one that defines a species, (vii) indicates the existence of some degree of heterogeneity among members of a species; among other definitions. The term species complex is commonly used to encompass several other concepts related to unclear species delimitation, such as cryptic species, sibling species and species flock (Bickford *et al.* 2007; Pinheiro *et al.* 2018). Despite that, species complex is widely used in botany, microbiology and zoology studies in past and recent publications, instigating discussions on the delimitation of species (Ando *et al.* 2005; Kwon-Chung *et al.* 2017; Scherz *et al.* 2019). It is usually defined after close examination of a given group by experts that detect uncertainty under a taxonomic name, referring to it as a complex in subsequent publications (Sousa-Paula *et al.* 2021). Therefore, the term is widely accepted by the scientific community as meaningful and valid for describing entities that cannot have their taxonomic boundaries precisely defined based on current knowledge and require special effort for their resolution. Furthermore, the designation of a group as a species complex has the secondary objective of drawing attention to the demand for its resolution in academic sectors.

Species complexes are products of varied evolutionary histories, occurring widely across the tree of life and being found primarily in species-rich habitats such as tropical rainforests (Bickford *et al.* 2007). Many of these taxa are at the beginning of their speciation process where hybridization may occur (Pinheiro *et al.* 2018). They may also be the result of evolution leading to at least superficially morphologically indistinguishable species (also known as cryptic or sibling species) or groups with extensive morphological diversity and little genetic divergence (derived by evolutionary radiation) (Vickery 1978; Bickford *et al.* 2007; Soltis & Soltis 2009). Understanding their evolution is crucial to better delimit the taxonomic boundaries within these groups, since species are fundamental units on which most research in life science is based (Sites & Marshall 2003). For applied research, correct species boundaries result in reliable biodiversity estimates as well as inform conservation strategies and natural area management approaches (Vogel Ely *et al.* 2017; Jha & Bhowmick 2021). Furthermore, the misidentification of species complexes related to economic, nutritious, or medical importance can result in negative consequences (Esterhuizen *et al.* 2013; Hendrichs *et al.* 2015). Plants are insufficiently researched for cryptic groups compared to animals (Bickford *et al.* 2007) and Solanaceae can be considered as a model family in this area due to its



various studied species complexes. The family also provides many examples of improvement in species delimitation of wild and domesticated taxa, as well as serving as evidence for the outcome of intricate evolutionary processes and artificial selection acting on complex taxonomic contexts.

Based on the above, our aim is to provide an overview of how studies involving species complexes in Solanaceae have been conducted. We quantified some general aspects that permeate species complexes in the family: (i) most studied wild and domesticated groups, (ii) sources of data, (iii) places where most studies are carried out, (iv) most recurrent scientific areas of study for solving species complex and (v) the frequency with which these studies support taxonomic decisions.

Materials and methods

We performed a systematic literature search in the Web of Science database (Institute of Scientific Information, Thomson Scientific) for articles published up to December 10, 2022. We used the following Boolean search: (Solanaceae) AND (complex OR species complex OR cryptic species OR biosystematic OR biosystematics OR sibling species OR integrative taxonomy OR iterative taxonomy OR species flock). Keywords were searched anywhere in the article (title, abstract, keywords, the main body of the manuscript, etc.). Additionally, we revised the six volumes of the International Symposium on the Biology and Taxonomy of the Solanaceae (Hawkes *et al.* 1979; D'Arcy 1986; Hawkes *et al.* 1991; Nee *et al.* 1999; Van den Berg *et al.* 2001; Spooner *et al.* 2006); the book "A Festschrift for William G. D'Arcy: The Legacy of a Taxonomist" (Keating *et al.* 2005); 59 volumes (1937-2022) of *Lilloa* journal; and 37 volumes (1961-2012) of *Kurtziana* journal. We used a broad definition of a species complex to broaden the number of articles selected, especially when considering older work. After screening all materials, a more in-depth search was performed to verify whether the studies really investigate a Solanaceae species complex from the perspective of at least one field of study.

We constructed a table to summarize the details of each study and compile the information (Tab. S1, Tab. S2). We recorded the genus studied in each article, as well as the focus group (i.e., species complex name, subgenus, section, a set of species of a given genus, etc.). We weighted the number of studies of each genus per species diversity using estimates of species per genera based on recent publications (e.g., Barboza *et al.* 2016; 2022; Knapp 2020; Martínez *et al.* 2023; Stehmann *et al.* 2009; Stehmann & Larocca 2023). Given the importance of domesticated taxa in Solanaceae and their role in the taxonomic complexity observed in the family, we also classified articles into "wild", "domesticated" or "both" wild and domesticated groups (naturalized taxa not used as crops were considered "wild"). We used the class "domesticated" for taxonomic groups selected by artificial selection, which

include mainly cultivated taxa. We checked if the articles collected their plant data *in situ* or *ex situ*. *In situ* studies made use of plants collected (or observed) in their natural habitat, *ex situ* studies used cultivated material, herbarium material and germplasm data in the research, while review articles were labeled "not applicable". Biogeographic realms (*sensu* Udvardy 1975) and countries were assigned to the location where the study took place, if explicit. We also checked if the article combined different methods to study the species complexes and reached a taxonomic decision (i.e., a clear statement regarding the taxonomic decision, likely followed by a detailed description of the decision and how the new circumscription is established). We checked which fields of study were explored, categorized as (adapted from Pinheiro *et al.* 2018): taxonomy (traditional methods using diagnostic qualitative characters), morphometrics (including phenetics), anatomy (including micromorphology), cytogenetics, reproductive biology (crossing-experiments, pollination and phenology), palynology, chemotaxonomy, ecology (use of bioclimatic data and niche modeling), population genetics, phylogeny, and phylogeography. Considering studies that used integrative approaches in their methods, we quantified the fields of study that were more frequently combined in a single study. Finally, we analyzed the proportion of use of the most frequent fields of study throughout the decades.

Results

Our survey identified 83 articles published between 1970 and 2022 involving species complexes in Solanaceae: 63 through Web of Science search (Tab. S3), 17 found in volumes of international symposiums of the family, two articles from the book dedicated to William G. D'Arcy (Keating *et al.* 2005) and one study from *Kurtziana* journal. The studies comprised nine genera: *Solanum* (55 studies), *Capsicum* (12 studies), *Petunia* Juss. (seven studies), *Nicotiana* L. (three studies), *Physalis* L. (two studies), and four more genera with one study each (*Cestrum* L.; *Datura* L.; *Deprea* Raf.; and *Witheringia* L'Hér.) (Fig. 1). Considering the relationship of studies per species diversity, *Petunia* (0.47) and *Capsicum* (0.28) were the most studied genera (Fig. 1). We found 45 studies involving only wild species, 35 combining wild and domesticated taxa, and three using only domesticated groups (Fig. 2). Fifty-seven studies used *ex situ* plant material, 18 used *in situ* plant material and four used both sources of data (Fig. 3A). Among the *ex situ* studies, cultivated plant material appeared in 28 of the articles gathered, germplasm 25 times and herbarium material 16 times (Fig. 3B). The Neotropical was the biogeographic realm with most studies (61), followed by Nearctic (15), Afrotropical (14), Indomalayan (8), Palaeartic (8), and the Australian (4) realms (Fig. 4).



Thirty-six studies (43.4%) integrated different methods and 22 (26.5%) made taxonomic decisions as one of the study results. The main fields of study were morphometrics (29 studies), populations genetics (23), reproductive biology (18), phylogeny (17), and taxonomy (14) (Fig. 5). For the integrative studies, we identified the combinations that appeared at least three times, resulting in the eight most common combinations presented in Tab. 1. Morphometrics was the field of study more often combined with other methods, appearing four times, followed by population genetics and reproductive biology, three times each (Tab. 1). The most frequent combination found in our survey is between morphometrics and reproductive biology (seven studies), while five other combinations were used in four studies each (Tab. 1). The first study on species complex dates back to the 1970s, and since then the use of population genetics has seen an increase in studies of species complexes in Solanaceae, while reproductive biology has seen a decrease (Fig. 6). Morphometrics and phylogeny have been continuously used since the 1990s (Fig. 6).

Discussion

Species complex studies by genus of Solanaceae

Solanum was the genus with the highest number of studies regarding species complexes, which is not surprising,

given its high natural and domesticated diversity, economic importance, and cosmopolitan distribution (Barboza *et al.* 2016). It is not only the most diverse genus of Solanaceae, but also ranks as one of the most diverse genera among all flowering plants (Frodin 2004) influenced, in part, by earlier revisions that included species then considered in other genera (e.g., *Cyphomandra* Sendtn., *Lycopersicon* Mill.) (Spooner *et al.* 1993; Bohs 1995; Peralta & Spooner 2000) and the constant rate of new species descriptions (e.g., Gouvêa *et al.* 2019; Gouvêa *et al.* 2020; Stehmann *et al.* 2020). Despite the recognition of many synonyms in some sections (e.g., *Solanum* section *Petota*), the number of non-resolved species complexes is still considerable (Miller & Spooner 1999). Pinheiro *et al.* (2018) also found *Solanum* as the most studied angiosperm genus in South America regarding species complexes in their native range. In our study, in which we included domesticated taxa of *Solanum*, the numbers were even more impressive, as 66.3% of the articles focused on at least one *Solanum* species complex, 45.5% of them (25 out of 55 studies) involving cultivated groups. The morphological complexity observed in *Solanum* species complexes is influenced by the domesticated taxa found in different biogeographic realms, since many studies seek to determine which wild progenitors gave rise to domesticated forms (Van den Berg *et al.* 1998; Gavrilenko *et al.* 2013). For the Neotropical realm, it is notable that many complexes comprise tuber-bearing taxa (section *Petota*). We highlight the case of *Solanum medians* Bitter

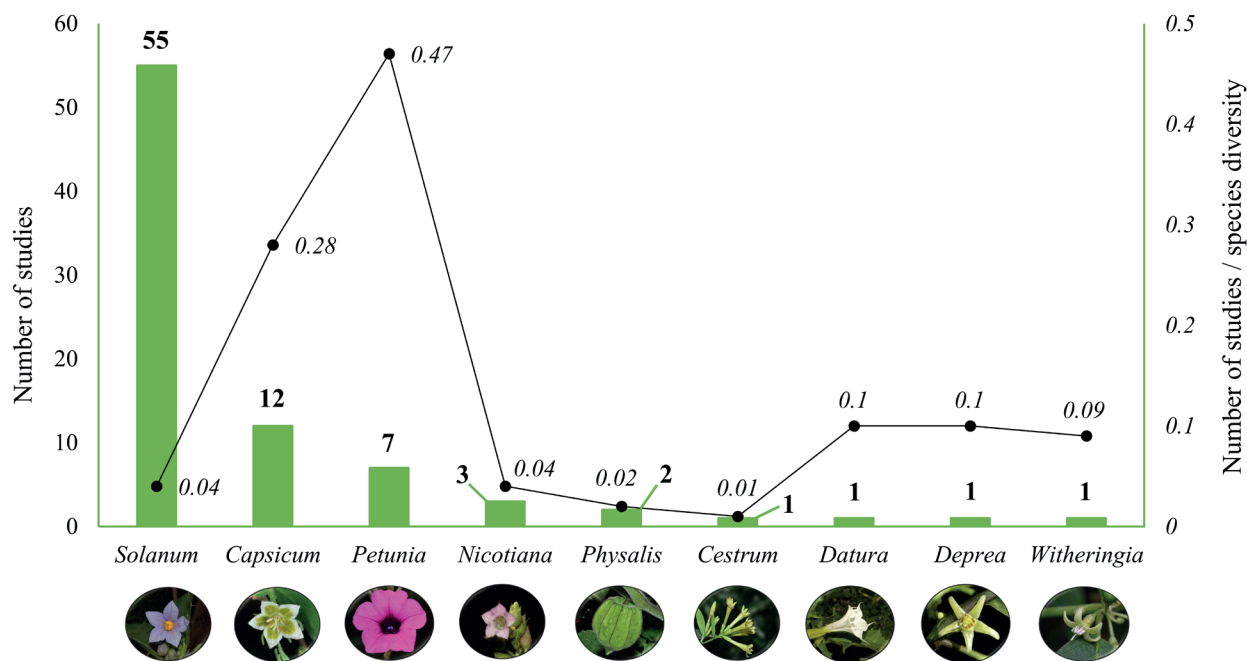


Figure 1. Number of studies (1970-2022) by genus in Solanaceae species complexes (left y-axis) and ratio between number of studies and species diversity of each genera (right y-axis). Species in the photos from left to right: *Solanum hexandrum* Vell., *Capsicum longidentatum* Agra & Barboza, *Petunia integrifolia* (Hook.) Schinz & Thell., *Cestrum parqui* L'Hér., *Nicotiana tabacum* L., *Physalis peruviana* L., *Datura stramonium* L., *Deprea abra-patriciae* (S.Leiva & Barboza) S.Leiva & Deanna, *Witheringia solanacea* L'Hér. *Deprea* and *Witheringia* photographs are courtesy of Rocío Deanna, while the others are courtesy of João R. Stehmann.

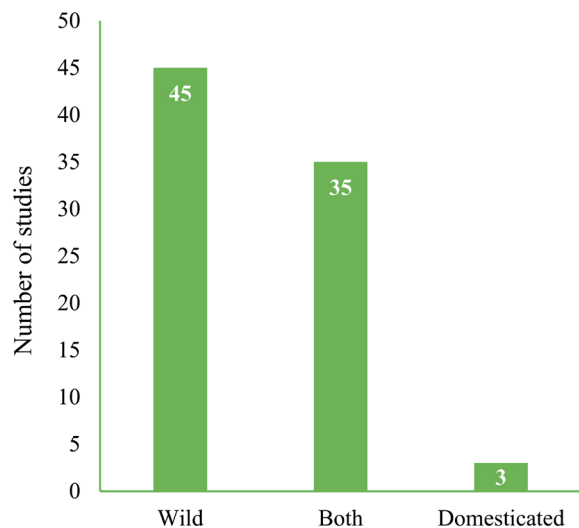
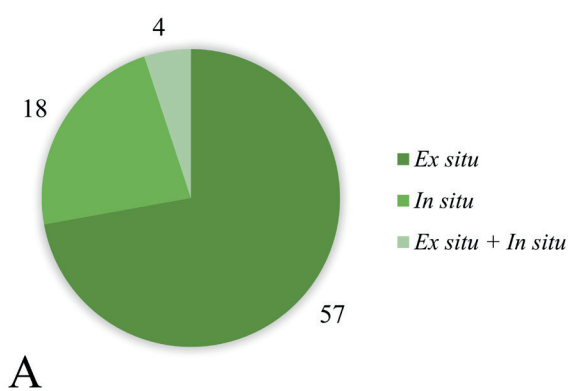
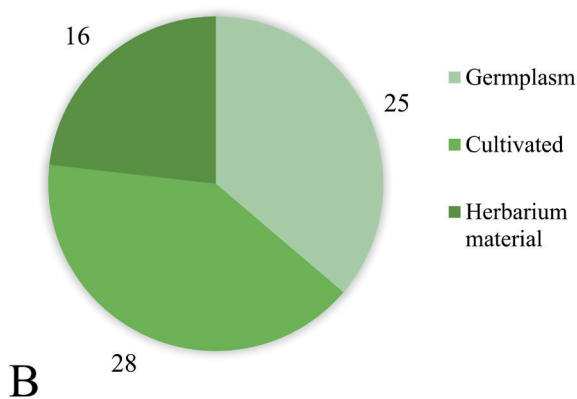


Figure 2. Number of studies using wild and domesticated species to study species complexes in Solanaceae (“both” indicates wild and domesticated species investigated in the same article).



A



B

Figure 3. A. Number of studies using *ex situ* or *in situ* data sources to research species complexes in Solanaceae (“not applicable” studies are not considered). B. Number of studies using different *ex situ* sources of plant material (“cultivated” includes material obtained by cultivators and studies that seeded wild species; some studies used more than one source, so the summing of graph B surpasses the *ex situ* value of graph A; all *in situ* studies uses data collected or observed from natural populations).

Table 1. Pairs of fields of study combined in three or more studies of species complexes in Solanaceae.

Fields of study integrated		Number of studies
Morphometrics	Reproductive biology	7
Morphometrics	Population genetics	4
Population genetics	Phylogeny	4
Morphometrics	Ecology	4
Reproductive biology	Taxonomy	4
Population genetics	Ecology	4
Morphometrics	Phylogeny	3
Reproductive biology	Cytogenetics	3

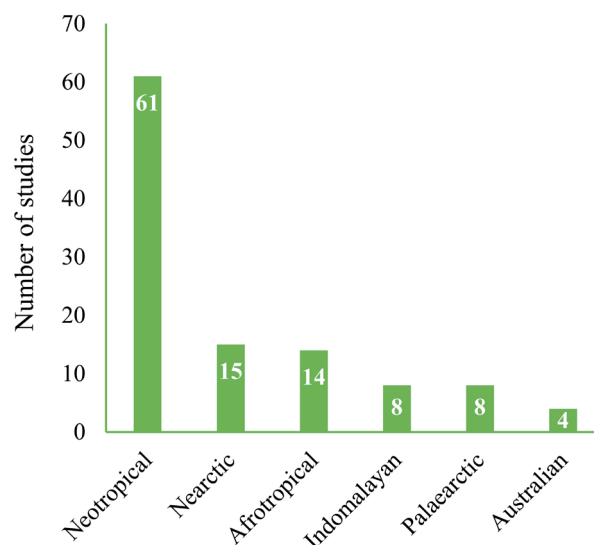


Figure 4. Biogeographic realms (*sensu* Udvardy 1975) considered in studies of species complexes in Solanaceae.

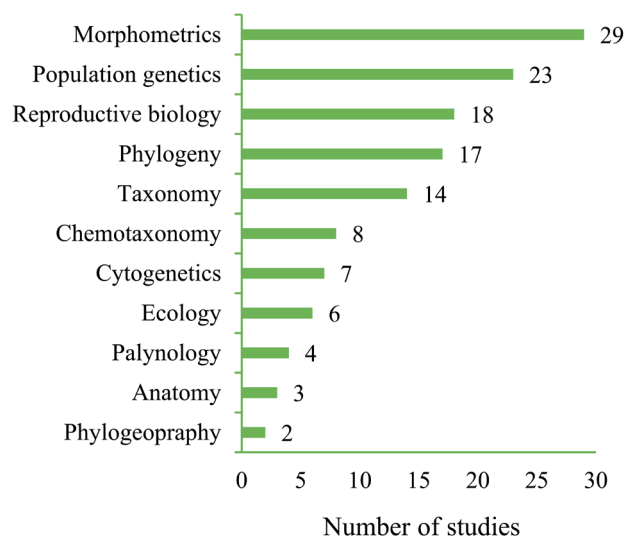


Figure 5. Fields of study used in research on species complexes in Solanaceae.



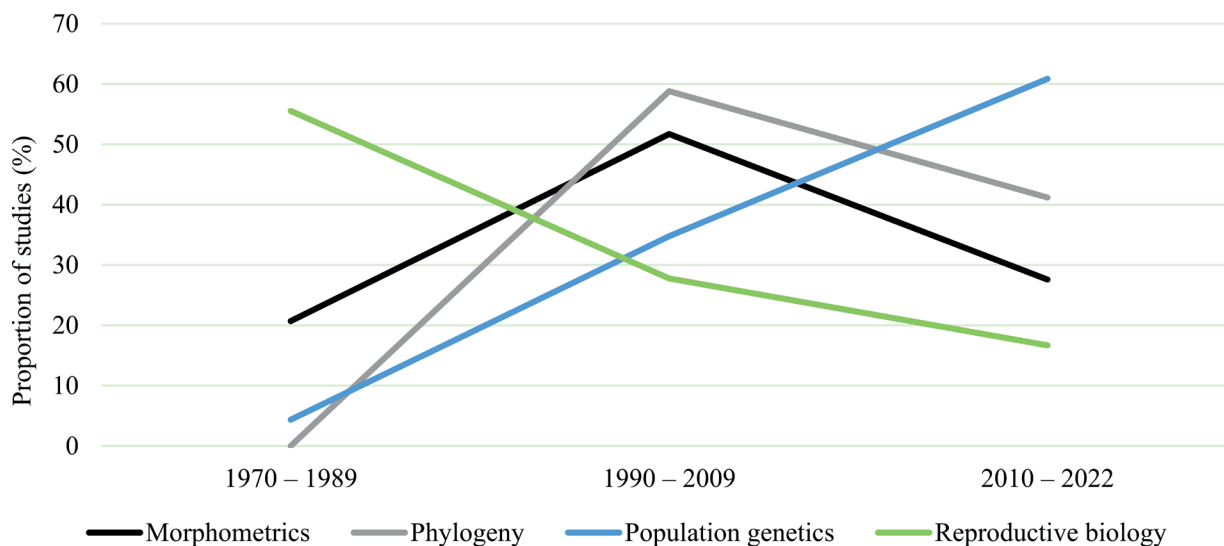


Figure 6. Proportion of studies of species complexes in Solanaceae for each of the four most applied fields of study over the years.

complex (Spooner *et al.* 2008) as a work that reinforces the trend for taxonomic reduction in section *Petota*. This study, conducted in Chile and Peru, provided morphometric evidence that allowed the authors to synonymize ten names under *S. medians* (Spooner *et al.* 2008). Still considering the Neotropical realm, *Solanum brevicaulle* Bitter complex, with wild and domesticated taxa morphologically similar to cultivated potato (*S. tuberosum*), was considered the putative progenitor of this important landrace (Van den Berg *et al.* 1998; Miller & Spooner 1999; Alvarez *et al.* 2008). Early work using morphometrics and molecular phylogeny confirmed the same findings of three groups within the *S. brevicaulle* complex, making it inevitable the collapse of the circumscription comprising 30 taxa (Van den Berg *et al.* 1998; Miller & Spooner 1999). Alvarez *et al.* (2008), applying morphometrics, found the same support for three groups, however, according to the authors “even these three ‘species’ had no species-specific characters and could only be distinguished with great difficulty because there was overlap of the best characters separating them”. The case of the *S. brevicaulle* complex demonstrates how challenging the resolution of species complexes can be, as at least three different teams analyzed the group, and yet no taxonomic decision has been firmly established. *Solanum* was the only genus with Afrotropical studies, where the *Solanum nigrum* L. complex was the main focus. This group forms a cosmopolitan species complex with many domesticated members with medicinal, forage or nutritional use in Africa (Dehmer 2001). *Solanum nigrum* complex has been studied under various approaches, such as anatomy (Gbile 1986), chemotaxonomy (Mohy-Ud-Din *et al.* 2010), molecular biology (Dehmer 2001; Manoko *et al.* 2007) and reproductive biology (Van Biljon *et al.* 2010), and still, there is considerable uncertainty regarding its taxonomy, in part due to its distribution across different continents.

The *Solanum melongena* complex is considerably studied in the Indomalayan realm, especially India, where we detected studies revisiting crossbreeding experiments (Khan 1979) and using chemotaxonomic methods (Pearce & Lester 1979; Haliński *et al.* 2011) as the focus. For the Australian realm, two studies involving the *Solanum petrophilum* F.Muell. and *Solanum clarkiae* Symon complexes, respectively, resulted in the description of new species through traditional taxonomic methods (Bean 2016a; b). There is plenty of *Solanum* material in germplasm from renowned institutes such as the Tomato Genetics Resource Center (University of California), the International Potato Center (Lima, Peru), and the US Potato Genebank (Sturgeon Bay, Wisconsin), which facilitates the development of studies with molecular data (Peralta & Spooner 2005; Hardigan *et al.* 2015).

Capsicum is an economically important genus because it comprises five species of pepper, each with different cultivars (Barboza *et al.* 2022). It is native to the Nearctic and Neotropical realms, but currently, India occupies a significant position in the production and consumption of chili peppers (Jha & Bhowmick 2021). All studies on *Capsicum* included domesticated taxa, ten out of twelve articles used *ex situ* material, and the main investigation with the genus is the evaluation of genetic variability between wild and domesticated forms (Albrecht *et al.* 2012; Thul *et al.* 2012). These genetic studies are related to the use of “species complex” as indicative of a gene pool. The most recent phylogenies of *Capsicum* established eleven clades within the genus, three of them (*Annuum*, *Baccatum*, and *Pubescens* clades) including domesticated forms (Carrizo García *et al.* 2016; Barboza *et al.* 2019; Barboza *et al.* 2020). Within these clades, we find the three complexes in the genus: *Capsicum annuum* L., *Capsicum baccatum* L., and *Capsicum pubescens* Ruiz et Pav. complexes (Silvar & García-González 2016). These complexes are somewhat historically



established within the circumscription of the genus and used as a type of infrageneric classification to indicate which wild species are related to peppers, and also which species are sexually isolated or can generate viable hybrids (Silvar & García-González 2016). The most recent revision of the genus also mentions the complexes to discuss aspects of some species (Barboza *et al.* 2022), mainly with regard to the clades *Annum* and *Baccatum*, which still present interspecific relationships pending resolution. Despite studies with *Capsicum* using an array of techniques and data, i.e., cytogenetics (Belletti *et al.* 1998), morphometrics (Pickersgill 1979), palynology (Martins *et al.* 2013), and reproductive biology (Onus & Pickersgill 2004), the majority of these works (11 out of 12) did not make taxonomic decisions. The “classificatory” use of species complex seen in *Capsicum* is common for microbial taxa, where they often correspond to groups that cannot be well-delimited based on DNA sequence data (Sharma *et al.* 2015; Kwon-Chung *et al.* 2017).

Articles on *Petunia* are notably unique, as most were carried out *in situ*, all covering wild species in the Neotropics, the natural distribution of the genus (Stehmann *et al.* 2009), and it appears as the most studied genus when weighting the results against its species diversity. *Petunia* also is the only genus with phylogeographic approach used to better delineate and study species complexes (Longo *et al.* 2014; Segatto *et al.* 2017) and the most studied genus using ecological data (bioclimatic variables and ecological niche) (Turchetto *et al.* 2014a; b; Segatto *et al.* 2017). We found studies for the *Petunia axillaris* (Lam.) Britton, Stern and Poggenb. and *Petunia integrifolia* (Hook.) Schinz and Thell. complexes, both interesting examples of using species complexes as models to better understand speciation in plants (Pinheiro *et al.* 2018). While the three subspecies of *P. axillaris* provided insights into evolution in the Río de La Plata grasslands and adjacent ecoregions (Turchetto *et al.* 2014a; b); the differentiation between coastal and continental populations of *P. integrifolia* formulated one of the first hypotheses of plant diversification driven by marine transgressions and regressions on the coasts of southern Brazil (Longo *et al.* 2014).

Nicotiana was represented in our survey by three articles, two in Australia and one in South America, the continents where the genus is more diverse (Knapp 2020). Australian works deal with the *Nicotiana benthamiana* Domin complex, traditionally considered as a model plant for plant-viral interaction studies due to the constant use of an accession called LAB (Chase *et al.* 2022). Genetic, molecular and taxonomic studies revealed a hidden diversity of five species under the name *N. benthamiana* as part of a project that aimed to document the diversity of the genus on the Australian continent (Cauz-Santos *et al.* 2022; Chase *et al.* 2022). Another article used geometric morphometrics and ecological data to, among other aims, better understand the relation of *Nicotiana forgetiana* Hemsl. and a putative new

species (Teixeira *et al.* 2022). Among the remaining five genera, three belong to the tribe Physalideae (*sensu* Barboza *et al.* 2016): *Deprea*, *Physalis* and *Witheringia*. The article on *Deprea* is a classic morphometric study that reaffirms sibling species as actually three different species using herbarium material (Sawyer & Rojas 1998). *Physalis* was the object of study in two articles conducted in Mexico and the United States of America, where the genus is more diverse (Sullivan 1985; Hudson 1986), both using an integrative approach. The study on *Witheringia* was the only one that combined phenology and pollination in an *in situ* work based on observation of natural populations (Bohs 2000). This study explored a hypothesis of hybridization as a factor that maintains the morphological similarities between two species through the analysis of phenological cycle and pollinator visitors, concluding a restriction of gene flow between the taxa. Both articles on *Cestrum* and *Datura* species complexes used herbarium material and, as a result, made taxonomic decisions, reestablishing the species *Cestrum mexicanum* Francey (del Castillo-Batista *et al.* 2017) and creating two varieties for *Datura stramonium* L. (Hassan & Amer 2019). The *Datura* study took place in Egypt, reflecting the now cosmopolitan distribution of *D. stramonium* originally native to Mexico and the US (Barboza *et al.* 2016), a trend usually only seen for genera with domesticated groups.

General aspects of species complexes studies in Solanaceae

Most of the studies were carried out in the Neotropical realm, which was expected given the high diversity of Solanaceae in South America, especially in the Andean region. Peru (19), Bolivia (16), Argentina (15) and Ecuador (12) were the countries covered by most studies. Northern Peru and southern Ecuador encompass the Amotape-Huancabamba zone, a biodiverse region characterized by a mosaic of environments resulting from the complex topography of mountains and rivers (Stern & Bohs 2010). This zone is considered a hotspot of endemism for some groups of *Solanum* (Knapp 2002). Furthermore, many *Solanum* wild potato species have been reasonably sampled in Peru, which probably also influenced the high number of species complexes studies (Spooner *et al.* 1999). In Bolivia, the center of diversity for groups like *Capsicum* (McLeod *et al.* 1982) and *Solanum* series *Circaeifolia* (Van den Berg & Groendijk-Wilders 1999), the Andean region registered the majority of studies. The number of studies in Argentina was also influenced by studies covering the Andes, with a significant number of studies on *Petunia* in the Río de La Plata region also elevating the importance of the country (Ando *et al.* 2005; Turchetto *et al.* 2014a; b; Segatto *et al.* 2017).

All studies covering the Nearctic realm also included the Neotropical realm, especially for complexes located around the US-Mexico border, such as some members



of the *S. nigrum* complex (Heiser *et al.* 1979). All studies involving the Indomalayan realm, except one, dealt with domesticated taxa, showing a remarkable trend of studies with species complexes in the region as having economic and cultural influence, mainly involving the *S. melongena* and chili pepper complexes in India. The studies carried out exclusively in the Afrotropical realm comprise four species complexes, among which only the *S. nigrum* complex awaits further research and resolution. It is evident the advance in knowledge regarding *Solanum* complexes in the region, as the *Solanum aethiopicum* L. (more specifically the section *Oliganthes* series *Aethiopica*), *Solanum incanum* L. and *Solanum macrocarpon* L. complexes were all studied and defined as a single species each (Lester & Niakan 1986; Bukenya & Carasco 1995; Olet & Bukenya-Ziraba 2001). We detected only two studies conducted exclusively in the Palaearctic realm: the aforementioned work on *Datura* (Hassan & Amer 2019), and a Saudi study that analyzed differences in the secondary metabolites of eleven species of *Solanum* from southwest Saudi Arabia, including the *S. nigrum* complex (El-Shaboury *et al.* 2017). We detected only four studies in the Australian realm, a region with high diversity of Solanaceae (Barboza *et al.* 2016). There are more taxonomically challenging *Solanum* lineages in the region than the ones covered by the two articles comprised in this review (e.g., Lacey *et al.* 2017), as well as Australian genera that need revision (e.g., *Cyphanthera* Miers and *Duboisia* R.Br.) (Barboza *et al.* 2016) and a great hidden diversity of *Nicotiana* (Chase *et al.* 2018), groups that may present species complexes awaiting detection. The remaining two realms were not covered by any article. This draws attention since the island of New Guinea (Oceanian realm) has a considerable number of native species of *Lycianthes* (Dunal) Hassl. and *Solanum* (Symon 1985; Knapp 2022), and New Zealand (Antarctic realm, Udvardy 1987) have much more naturalized than indigenous nightshades (Howell & Sawyer 2006; Lange & Rolfe 2010).

About a third of the studies used morphometric approaches to investigate species complexes, used consistently since since the 1970s. This is certainly linked to its accessibility, where data can be obtained by measuring herborized material and analyzed using accessible statistical procedures, such as univariate and multivariate analyses (Henderson 2006; Ezard *et al.* 2010). Still, it is clear the importance botanists place on morphological data when investigating difficult groups, as size and shape disparities are commonly the first ones observed, providing evolutionary insights (Stuessy 2009). The use of molecular markers has increased significantly in the recent decades influenced by technological advances. Its adoption in population genetic studies generally aims to investigate genetic diversity and variability to identify kinship between wild and domesticated taxa (Albrecht *et al.* 2012; Gavrilenko *et al.* 2013); while phylogenetic works usually analyze the relationship of members of a species complex and/or try to

place them within a clade, especially for *Solanum* (Miller & Spooner 1999; Spooner *et al.* 2007; Särkinen *et al.* 2015). Studies analyzing reproductive aspects of species complexes were more often applied in past decades, especially using crossing experiments to test compatibility and hybridization (Rick 1986; Omidiji 1986; Olet & Bukenya-Ziraba 2001). Cytogenetic studies have generally explored the ploidy of wild and domesticated groups and defined groups based on DNA content (Bukenya & Carasco 1995; Belletti *et al.* 1998). Given the chemical diversity of Solanaceae, chemotaxonomic studies involving species complexes were also relevant, focusing on secondary metabolites (El-Shaboury *et al.* 2017), protein analysis (McLeod *et al.* 1979; Pearce & Lester 1979), flavonoids (Sullivan 1985), and comparison of alkaloidal profile (Mohy-Ud-Din *et al.* 2010). We also highlight the use of traditional taxonomic methods in recent years to support taxonomic decisions (Bean 2016a; b; Chase *et al.* 2022). Few studies (6) used ecological approaches, e.g., bioclimatic variables and niche modelling. These data are quite useful to disentangle taxonomic complexity into specific and infraspecific categories, given the impact that environmental conditions and distribution have on the evolutionary process driven by spatial isolation (Stuessy 2009). Ecological analyses require years of experience and training, but they can be relatively inexpensive compared to other methods. Furthermore, in recent years, the availability of big data on plant distribution and climate, such as GBIF (<https://www.gbif.org/>) and WorldClim (Fick & Hijmans 2017), has helped to refine the discussions about the ecological niche of species and the impact that climate has on phenotypic plasticity. Palynology (4), anatomy (3) and phylogeography (2) were the least applied fields of study, probably due to the need for an interdisciplinary team working on systematic research involving these areas. These three fields of study are also quite expensive and require experience to conduct them (Stuessy 2009). Other expensive areas, such as phylogeny and population genetics, are probably much more used in species complexes studies due to their more direct link with systematics in line with the availability of databases such as GenBank (<https://www.ncbi.nlm.nih.gov/genbank/>) and TreeBASE (<https://www.treebase.org/>).

Less than half (43.4%) of the articles applied a combined approach to study species complexes. The most common fields of study in integrative studies were cytogenetics, ecology, morphometrics, phylogeny, population genetics, reproductive biology and taxonomy. Given the complexity of delimiting species complexes, a taxonomic assessment that considers different lines of evidence and methods tends to result in a more substantiated circumscription proposal (Dayrat 2005; Padial *et al.* 2010). Although the use of integrative approaches is not a universal solution for species delimitation (Padial & De La Riva 2010), for cryptic lineages specifically it is gaining popularity, especially for groups where previous works have not reached a reliable



hypothesis at the species level. The study by Raduski & Igić (2021) is an example of this tendency, where five methods were integrated (ecology, morphometrics, phylogeny, population genetics, and reproductive biology) to investigate the taxonomic status under the name *Solanum chilense* (Dunal) Reiche. They concluded that the group comprises at least two species represented by coastal and Andean populations, plus a possible third species awaiting closer examination. Ispizúa *et al.* (2015), using cytogenetics, morphometrics and reproductive biology in wild potato species with considerable morphological variation, were able to better understand hybridization patterns, gene flow barriers and differences in ploidy. The authors found complex mixtures of parental genotypes and hybrid generations. This study is an example that the existence of species complexes does not prevent their use in research that does not seek taxonomic resolution.

Around three-quarters (73.5%) of the studies analyzed did not change or confirm taxonomic status, a result we did not necessarily evaluate negatively. Given the way biological knowledge is built, any new information about cryptic lineages could be potentially crucial for future taxonomic decisions and many articles discuss the support (or lack of) of groups, indicating the next steps. Jacobs *et al.* (2011), for example, explicitly show a lack of support for at least 43 species in *Solanum* section *Petota* using population genetic tools. They argue that combining their findings with morphological, geographical and reproductive data in future assessments could result in a more reliable taxonomic decision. The results of Cauz-Santos *et al.* (2022) were also crucial to the taxonomic decisions made by Chase *et al.* (2022) on the *N. benthamiana* complex, among many other examples of information gathered to support changes in circumscription (e.g., Miller & Spooner 1999; Spooner *et al.* 1999; Turchetto *et al.* 2014b; Raduski & Igić 2021). Another interesting outcome particular to Solanaceae is the classification of domesticated taxa, such as the detailed description of potato cultivars by Huamán & Spooner (2002).

Many of the studies in our survey were partially economically motivated, collecting information to improve the agronomic performance of crops. Through these articles, it is evident that biotechnological and genomic approaches targeting domesticated groups end up contributing to a better understanding of the biology of lesser-known, wild and native relatives, especially for *Solanum* and *Capsicum*. Still, for most Solanaceae generic lineages that do not have significant economic importance, studies of species complexes are scarce. Our survey detected studies with species complexes comprising less than 10% of the diversity of Solanaceae genera. Even if we consider the possibility that many genera are well-researched and do not contain species complexes, the number is still unexpectedly low. *Cestrum* contains 150-200 species and we detected only one study, while *Lycianthes* comprises around 150 species

distributed throughout America, Asia, Australia and Pacific islands (Knapp 2022) and was absent in our revision. Their considerable diversity raises questions about the existence of undetected species complexes. However, although less likely, smaller genera also need to be revised, like the case of the monotypic *Metternichia* J.C. Mikan, where enough evidence has been gathered to support the splitting into two species (LS de Souza *et al.* unpubl. res). Finally, we also point out the many complicated groups not clearly defined as species complexes in Solanaceae. One compelling case is *Solanum cylindricum* Vell., a member of the section *Cyphomandropsis*, revised by Bohs (2001). The author recognized an extensive morphological range in pubescence, leaf size and shape, and inflorescence and fruit variation, resulting in six different “morphotypes” that she could neither clearly consider as different taxa nor assert strong support for maintaining it as a single species. This morphological disparity could potentially indicate a species complex under the name *S. cylindricum*, requiring further investigation.

Conclusion and future perspectives

Overall, we observe advances and increasing knowledge with respect to species complexes in Solanaceae but, to enable further progress, more genera need to be revised, especially considering the high diversity in the Neotropics. We highlight the *S. nigrum* complex as probably the most challenging unresolved species complex in the family today, requiring an international and interdisciplinary team effort to achieve the best circumscription for the group, given its cosmopolitan distribution. We also encourage consulting monographs to detect potential species complexes, as many groups may have been reviewed over a decade ago. Finally, we stimulate the use of ecological, palynological, anatomical and phylogeographical data and techniques, given their potential relevancy in showing key distinctive characters within species complexes.

In the first chapter of the volume based on the first edition of the “International Symposium on the Biology and Taxonomy of the Solanaceae” (Hawkes *et al.* 1979), D’Arcy stated: “There are important gaps in our knowledge of the Solanaceae. Several large genera have never been revised, and many have no revisions for more than limited portions of their ranges”. More than four decades later, we can say without a doubt that some of these “many gaps” have been filled, given that many genera and species of the Solanaceae family were reviewed and discovered. Also, the advent of molecular studies improved our understanding of the phylogenetic relationships within the family and allowed the elaboration of more grounded systematic hypotheses. All this body of work shows that the still poorly understood and unknown species complexes in Solanaceae will eventually be resolved, gradually elucidating more aspects of this fascinating family.



Acknowledgements

We thank Maycon L. da Luz (Biological Sciences library, UFPR), Miriam del V. Cuasollo (Imbiv library, UNC) and Rocío Deanna (CONICET, UNC) for digitalizing articles from the Kurtziana and Lilloa journals. We also thank Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) for the master's fellowship granted to LSS associated with the Graduate Program in Plant Biology at UFMG; and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for the research productivity grant to JRS (APQ 311416/2021-7).

Conflict of interest

The authors declare no conflict of interest.

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