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New insights into the species problem

YANG Yong[1*] & ZHOU ZheKun[2]

[1] *State Key Laboratory of Systematic and Evolutionary Botany, Institute of Botany, Chinese Academy of Sciences, Beijing 100093, China;*
[2] *Key Laboratory of Biodiversity and Biogeography, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, China*

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Historical opinions of the "species problem" are briefly reviewed, and four salient stages are recognized according to origin of species concepts. We propose that species is the unit preserving superior gene assembly and is maintained by specific mechanisms. Based on characteristics of plant evolution, we assume that understanding plant species may include three stages, i.e. morphological recognition stage, multidisciplinary verification stage, and illuminating mechanisms preserving superior gene assembly.

species concept, plant, evolution, phylogeny

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Heredity and variation are fundamental features of living beings. Life on earth has a venerable history of 3500 millions of years, and has experienced an evolutionary process from lower to higher, from simple to complex, from water to land [1,2]. Evolution of life refers to descent with modification, and includes two essentially independent processes, i.e. transformation of characters in time and genealogical diversification. During this long evolutionary process, the struggle for life in the living world was such that individuals with nonadvantageous variation were eliminated while those with advantageous variation were preserved through natural selection. Evolution of life lies in the filtration of genetic variation through natural selection, resulting in stepwise accumulation of advantageous variation accompanied by extinction of deleterious variation, by which discontinuous groups of life have evolved. According to the extent of discontinuity, taxonomists rank these groups of life within a categorical system where 'species' is the fundamental unit of classification. Species is the most fundamental presence of biodiversity in nature, and is the basic unit for study in most biological disciplines [2–7]. Although most biologists know what is a species when they mention the word 'species', it is difficult to accurately determine the species concept because well accepted criteria are absent and controversies about the criteria are collectively referred to as the "species problem" [7–14].

It is essential to not only theoretically but also practically discuss and explore the species concept. First, the species problem is one of the most fundamental questions in biology [15], and remains unresolved. Theoretically, the importance of species in biology is comparable to the role of cells in the constitution of organisms [7]. The species problem is the first question to be answered in the seven primary questions of systematic biology [4]. Second, the species problem is not only a theoretical problem, but also a practical problem which is significant in many fields of biology [16]. In this sense, the subjectivity of the species concept needs to be reduced to the minimal extent for practical manipulation, which makes identification accurate, normative, and convenient. To solve the practical difficulty of identifi-

*Corresponding author (email: ephedra@ibcas.ac.cn)

cation, international colleagues have moved towards demarcating species using a DNA barcode [17–19]. However, this project depends upon a similar criterion problem, the differentiation of DNA or morphology. The core of this problem remains the species problem.

We review the history of the species concept, analyze different theoretical backgrounds of the species problem to understand the key issues involved in the species concept, and try to generate a practically feasible model of the species concept in combination with the characteristics of plant evolution. Our goal is to arouse international attention to the species problem and facilitate its settlement.

1 The species concept and its key issues

Many concepts concerning species were proposed in history. Mayr [8,20], Davis & Heywood [21], Tong [22], Hsu [23], Zhao [24], Ni & Li [25], Lee [26], Stuessy [27], gave a brief review, and Mayden [10] collected 22 concepts of species. At the end of the last century, debates about the species concept reached a climax [28,29]. The Biological species concept [30–32], the Hennigian species concept [33–35], the Phylogenetic species concept [36–38], and the Evolutionary species concept [39–41] were proposed and developed in this period. Recently, other biologists discussed the species concept from their own point of view, e.g. Wu [13] and de Queiroz [7]. Clarification of the theoretical backgrounds of different species concepts is necessary in order to understand such a diversified and complicated problem as the species concept. Investigation of the theoretical background may be helpful for finding the key to solving the species problem so that it might be possible to judge which species concept is more reasonable than the others.

Evolutionary biology has undergone three important innovations, and each innovation had an impact on the development of the species concept. The first innovation is represented by Darwin's "*Origin of Species by Means of Natural Selection or the Preservation of Favoured Races in the Struggle of Life*". Darwin [42] claimed that all living species were derived from a common ancestor driven by a process called natural selection. This opinion fundamentally changed the Essentialist species concept and the Creationist interpretation of species dominant at that time. The second innovation is represented by the Modern evolutionary synthesis upheld by Mayr and Dobzhansky. The Modern evolutionary synthesis integrated genetics into evolutionary biology, and elucidated the role of natural selection in the process of evolution from a genetic viewpoint. The third innovation is Hennig's cladistics which introduced mathematical methods into evolutionary biology, which developed evolutionary biology in theory and methodology. Each innovation in biological thinking provided fundamental theoretical bases for the development of species concepts. According to an analysis of known species concepts, the development of their theoretical bases is divided into four phases. First, the Typological species concept was leading in debates before publication of Darwin's *Origin of Species*. Second, the Morphological species concept was dominant in the period from the publication of Darwin's *Origin of Species* up to the Modern evolutionary synthesis. Third, Mayrs' biological species concept (BSC) was the leading concept after the Modern evolutionary synthesis but before Hennig's cladistics. The biological species concept tried to elucidate the biological cause of the presence of species and the mechanisms of speciation. Fourth, the Phylogenetic species concept elucidated the relationships of species [10].

The theoretical roots of species concepts in each phase are detectable. The Typological species concept was dominant before the publication of Darwin's *Origin of Species* when researchers were looking for the invariant pattern of species. The *Origin of Species* shook the unchanging belief concerning species, and proposed the idea of homology and common ancestry and natural selection as driving forces of evolution, recognizing continuity of variation and delimited morphological species using discontinuity. Integration of genetics and evolutionary theory was the hallmark of Modern evolutionary synthesis which tried to elucidate evolutionary phenomena and mechanisms using genetics, and advocated the Biological species concept that claimed reproductive isolation as the way to protect the genetic pool. Innovation of cladistics in theory and methodology initiated a series of new species concepts, e.g. the Cladistic species concept, the Phylogenetic species concept. A few new species concepts were developed in each of the four phases, coexisting with old species concepts, and new versions of old species concepts were developed in the process of debates with new species concepts. Integration of different disciplines promotes new species concepts or develops new versions of old species concepts, for an example, studies in molecular biology supports new species concept [13]. Data accumulation may result in a new theoretical synthesis in the future.

1.1 Controversy before Darwin

The ancients did not recognize the biological integrity of species, e.g. Aristotle and Theophrastus believed that the seeds of one species of plant could germinate into plants of another species [8]. Because of the uncertainties about the nature of species, no consistent terminology existed, with the same term being used in totally different meanings in different contexts. It wasn't until the 16th century that the usage of the term 'species was fixed in the religious Reformation when the fixity and complete constancy of species became a firm dogma [8]. Genesis required belief in the individual creation of every species of plants and animal on the days prior to Adam's creation. The species thus were the unit of creation. Most of the herbalists in their studies of wild plants likewise arrived at the idea that species were well-defined units of nature and that they were constant and sharply separated from each other [8].

The Essentialist species concept as the creationist interpretation of species of the Christian fundamentalists maintains that each species is characterized by its unchanging essence and separated from all other species by a sharp discontinuity, all those objects belonging to the same species that share the same essence [8]. As a result, the term 'species' is applied to both living beings and such inanimate objects as minerals. The presence of the same essence is inferred on the basis of similarity such that species were defined as groups of similar individuals that are different from individuals belonging to other species [8]. The Essentialist species concept was almost unanimously accepted by post- Linnaean taxonomists (e.g. Charles Lyell, Michel Adanson). This concept postulated four species characteristics: (i) species consist of similar individuals sharing in the same essence; (ii) each species is separated from all others by a sharp discontinuity; (iii) each species is constant through time; and (iv) there are severe limitations to the possible variation of any one species [8,25]. Although Buffon preliminarily proposed criteria of reproductive isolation for the delimitation of species, he believed species to be constant and invariable [8]. The Typological species concept of Linnaeus is close to the Essentialist species concept, maintaining that any one species is an entity different from other species by means of a set of diagnostic features, but it is totally subjective to determine diagnostic features. The so-called Typological species concept is actually not a concept but an artificial approach for delimiting species. The results using this method are some natural groups without biological attributes.

The Nominalistic species concept is a similar contemporaneous concept. Nominalism, a medieval school of philosophy, rejected the notion of essentialism that similar things share the same essence, and claimed instead that all that classes of similar things share is a name, only individuals exist while species or any other classes are man-made constructs. The Nominalistic concept of species remained popular among botanists throughout the nineteenth century [8,25].

1.2 Debates from Darwin to the modern evolutionary synthesis

Darwin's *Origin of Species* is a milestone in the history of human thought, which contributed to evolutionary biology in two primary aspects. First, any given species is variable (evolution), natural selection is the driving force for the evolution of life. Second, it maintains the principle of common ancestry that all descendants in a clade were derived from a common ancestor. The two points have not been surpassed 150 years after publication of the book. Darwin did not give a concrete concept of species although he named his influential book *Origin of Species*. Darwin's species concept is based on morphology believing that "in determining whether a form should be ranked as a species or a variety, the opinion of naturalists having sound judgment and wide experience seems the only guide to follow" [42]. As a result, it seems that Darwin has no objective and well-accepted criteria in determining species. His species concept in *Origin of Species* is the morphological species concept based only on degree of morphological difference. The categories species and genus only indicate their inclusive extent but have no fundamental difference. For instance, a family includes multiple genera and a genus includes multiple species, indicating that the category family is larger than the category genus and the category genus is larger than the category species only in the inclusive extent, and the difference between families is larger than the difference between genera within a family, and the difference between genera is larger than the difference between species. It is worth considering why Darwin named his book *Origin of Species* as he apparently believed that no fundamental difference exists between the categories species and genus.

Mayr [8] mentioned that Darwin changed his species concept from an early concept similar to the Biological species concept to the Morphological species concept. The Morphological species concept in Darwin's *Origin of Species* has been used until the present, e.g. the taxonomic species concept of Cronquist [43] and the Phenetic species concept of Sokal & Crovello [44] all agreed that species is the minimal group of living beings bearing features continuous within a group but discontinuous between groups and is readily identifiable using common approaches. The species concept currently used in plant taxonomy remains based on morphological differences.

1.3 Debates from the modern evolutionary synthesis to cladistics

Although the morphological species concept in Darwin's *Origin of Species* implied that variation is continuous within a species but disjunct between species, it does not answer why living beings exist in the form of species in nature. Modern evolutionary synthesis integrated genetics into evolutionary theory, and explained evolutionary phenomena and speciation. Several researchers who pushed the debates of species concepts forward were Mayr, Stebbins, Dobzhansky, and Simpson. Several entomologists from the end of the 19th century to the beginning of the 20th century proposed the Biological species concept, Mayr's [20] Biological species concept is the most classic, and became the leading concept in the following debates of species concepts in the 20th century.

Mayr [20] developed the early species concept of Buffon, and believed that species are groups of actually or potentially interbreeding natural populations which are reproductively isolated from other such groups. A population is a reproductive community of individuals which share in a common gene pool and live in a certain time and space [15]. A species consists of one or more such populations. The biological species concept proposed a new criterion for delimitation of species, i.e. reproductive isolation [20,45]. Reproductive isolation is a concept from population genetics, indicating that no genetic exchanges occur between two species in contact with each other. The stop of genetic flow is the result of genetic attributes of the two species, and is not caused by external barriers. In addition, Mayr [20] believed that species are non-dimensional, but a concrete species is multi-dimensional. The appeal of the biological species concept lies in its simplicity, its agreement with the Neo-Darwinian emphasis on gene flow and allopatric speciation, and its testability [46].

The biological species concept answers two fundamental questions of species. First, why do living beings exist as species in nature? Mayr believed that the biological meaning of species is to preserve superior gene pools. There are two ways to preserve gene pools, one is via sexual species, the other is via asexual species (Agamospecies). Second, species exist under the condition of reproductive isolation. Mayr [8] later thought that this biological species concept is imperfect, and believed that species are not only reproductively isolated communities but occupy certain ecological niches in nature. As a result, reproductive isolation and ecological niche are two definitive features of species. At the same time, Mayr emphasized that the reproductive isolation criterion is special to sexual species, and the ecological niche criterion is applied to the Agamospecies because the reproductive isolation criterion is not applicable under such circumstances. Mayr & Ashlock [45] returned to his earlier species concept. Other species concepts that stress genetic exchanges are the Isolation species concept, the Genetic species concept, the Cohesion species concept, and the Ecogenetic species concept [10,47].

The Biological species concept is not ideal. First, the Biological species concept is special to sexual species but is not applicable to agamospecies [8,23,48]. Thus, the Agamospecies concept is parallel to the Biological species concept. The Agamospecies concept can't be applied to biparental sexual species but only to uniparental asexual living beings. Agamospecies are usually the result of interspecific or intergeneric hybrids which may give rise to gametes but can't be fertilized. The agamospecies may be part of a species complex which includes both sexual and asexual species. These species usually have quite confined distribution. Second, the reproductive isolation criterion is difficult to use, and much less convenient than morphology. Third, genetic flow is in fact present between many distinct species of plants, but they nonetheless conserve their own specific traits, for which the Biological species concept is unable to provide a reasonable explanation. Because of these reasons, though adherents proposed new explanations for the Biological species concept to reconcile difficulties [49], many other species concepts were developed, e.g. the Evolutionary species concept [50], and the Gene species [13].

Another species concept in Modern evolutionary synthesis was proposed by Simpson [50] who described species from an angle of origin based on palaeobotanical evidence. The Evolutionary species concept consists of genealogy from ancestor to its descendant populations having a unidirectional evolutionary trends. Species differ from each other by morphological characteristics or time, which shows strong subjectivity, and weakens the fundamental questions of the species concept. Examples of other similar concepts are the Successional species concept, the Palaeospecies concept, the Chronospecies concept, and the Phyletic species [2,22,10].

1.4 Debates since Hennig's cladistics

Hennig's [51] cladistics introduced rigorous logistic methods into evolutionary biology which altered systematic biology not only in terms of methodology but also in terms of theory and laid the foundations for the tree of life [24]. The term 'species' was subsequently redefined according to the principles of cladistics in combination with molecular data. Cladistics laid emphasis on monophyly and cladogenesis. Monophyly is a clade consisting of an ancestor and all its descendants. The process during which an ancestor is divided into two sister species is called cladogenesis.

The Cladistic species concept suggests that species is a lineage, which includes three kinds of situation. First, it indicates a group of living beings between two speciation events, e.g. intermodal species. Second, it suggests a group of living beings between a speciation event and an extinction event. Third, it implies a group of living beings derived from a speciation event [10].

The Hennigian species concept indicates that species is a reproductively isolated natural population or group of natural populations which originated via the dissolution of the stem species in a speciation event and ceased to exist either through extinction or speciation [33]. This notion was derived from an earlier version of Hennig's species concept and further developed by incorporating reproductive isolation into Hennig's species concept by Meier & Willmann [10].

With the development of phylogenetic systematics, some researchers need an operational lineage definition of species which is process-free, which led to the Phylogenetic species concept including three versions, i.e. the Autapomorphy version, the Diagnosable version, and the Genealogical version. The criterion of the Autapomorphy species concept stipulates that a species contains all of the descendants of one ancestral population and is identifiable by autapomorphies [46,53]. The Diagnosability species concept requires diagnosability and defines a phylogenetic species as the smallest aggregation of populations (sexual) or lineages (asexual) diagnosable by a unique combination of character states in comparable individuals, this concept which is based on characteristics, proposed by Nixon & Wheeler [54]. A third criterion for the Phylogenetic species concept, proposed by Baum & Shaw [55], is basal exclusivity stipulating that members of a group be more closely related to one another than to any organisms outside the group. Individuals of a species are deemed more closely related to one another than to organisms from another group if their genes share more recent common ancestral genes than they do with individuals in any other group. Judd *et al*. [46] commented that this concept is potentially flawed in that different genes often give different patterns of coalescence.

The species concept at the genic level is again distinct from the Biological species concept. Differentially adapted concept or Genic species indicates that species are groups of living beings that are differentially adapted and upon contact are not able to share genes controlling these adaptive characters, by direct exchanges or through intermediate hybrid populations [13]. The Genic species concept allows genetic exchanges between species which preserve a certain set of gene assemblies. The Biological species concept stresses reproductive isolation and any genetic exchanges between species would break the integrity of the genome of a species, while the Genic species concept suggested that the reproductive isolation is a byproduct of differential adaptation. The Genic species concept thus is different from the Biological species concept but resembles Darwin's view of speciation, i.e. species are groups of organisms differentially adapted to certain kinds of environments. Noor [56] agreed on the observation of Wu [13], but insisted that it is not the time to revise the Biological species concept. The Genic view of the species concept opened a new perspective for the study of speciation mechanisms, and provided a new way of thinking about the species problem [57]. Although the complicated mechanisms of speciation remain ambiguous [57,58], it is clear that the Genic species concept partially explained why organisms exist as species in nature comparing to the Biological species concept. According to Wu [13], any given species is not for the preservation of gene pools but for the preservation of a certain gene or a certain set of genes, with any subsequent reproductive isolation as a byproduct of differential adaptation. This recognition seems consistent with the actual situation with regard to plants.

De Queiroz [7] tried to propose an inclusive species concept that species are metapopulation lineages (the Metapopulation species concept). A metapopulation is a group of populations in Mayr's Biological species concept. It is clear, however, that this species concept doesn't follow Mayr's non-dimensional principle of species.

1.5 The key of the species concept

1.5.1 The duality of the species concept

It is not difficult to note from previous debates that the species problem actually touches two questions, first is the species category, while the second is the species taxon. The two questions should not be confused and former researchers chiefly focused on the species category [3,5]. A few researchers believed that the species problem included three aspects, i.e. the species concept, the species category, and the species taxon [59]. The species category is an abstract notion, and is the core of the species problem, containing all of the species in nature. By contrast, the species described in taxonomic practice are scientific hypotheses to be tested, only those valid hypotheses repeatedly tested are 'good' species, e.g. *Ginkgo biloba* L., *Metasequoia glypostroboides* Hu & Cheng, *Eucomia ulmoides* Oliv. Experienced botanists would likely find that there are certain distinct species in a genus while many other species in the genus make up a species complex due to their variable characters. Those distinct species often withstand repeated tests and thus are good species, e.g. *Ephedra rhytidosperma* Pachomova, *Ephedra triandra* Tul., *Lindera glauca* Blume, *Quercus variablilis* Blume. We believe that each such good species corresponds to a valid scientific hypothesis. There are over 30000 species of higher plants in China, which correspond to more than 30000 hypotheses, though most of these hypotheses need further testing.

1.5.2 The biological meaning of species

The primary question in the discussion of the species concept concerns why species exist in nature. Another way to view that question is to consider why natural selection produced the discontinuity between species and why it didn't support a continuous entity of all living beings. This actually is the biological meaning of species [32]. The basis of the Biological species concept is that species preserve the gene pool and any genetic exchanges between species would break the reproductive isolation of species. However, recent molecular biology suggested a different scenario that species is the manner to preserve or conserve superior genes or gene assemblies wherein certain kinds of genetic exchanges between species do not influence the presence of species if these genetic exchanges do not change the superior gene assembly [13].

1.5.3 Species as a concept with many attributes

Many species concepts have been proposed, some emphasize mechanisms, e.g. the Biological species concept, the Recog- nition species concept, the Cohesion species concept, and the Ecological species concept, others stress history, e.g. the Evolutionary species concept, the Diagnosability species concept, the Genealogical species concept, and the Phenetic species concept [60]. These species concepts share a common feature which is that all of these species concepts are based on certain fundamental biological attributes of species, for example, the Morphological species concept is based on the morphological characteristics of species, the Evolutionary species concept is based on the temporal characteristics of species, the Biological species concept is based on the reproductive characteristics of species, the Ecological species concept is based on the ecological characteristics of species, while the phylogenetic species concept is based on phylogenetic traits of species (monophyly, diagnosable, or basal exclusive).

A concrete species as an entity present in nature is a multidimensional entity with temporal, spatial, and characteristic attributes, thus the species taxon has multiple attributes. Because species are an objective presence, and knowledge tends to be the subjective reflection of an objective reality, it is understandable that contingency may exist between the subjective reflection and the objective thing, as cognition may be incomplete or inaccurate. Understanding of objective reality is similar to taking a part from the whole. In practice, researchers frequently considered the species concept to be equivalent to the species taxon, which might be the cause of the presence of the species problem.

Species is an abstract notion, and is a reproductive unit, an evolutionary unit, or a taxonomic unit [2]. A species is an entity of objective presence in nature, a group of living beings extending in time and space, which bears a series of characteristics. Difference may be present in one or more aspects between different species, e.g. reproductive features, morphological features, evolutionary characteristics, ecological attributes. Differences between different species generally are not ascribed to any one certain kind of character, but they are perceived through certain ways including unaided eyes. In other words, those recognizable differences of entities must be differences between objective species, while those potential differences between species may not have been recognized, e.g. those incipient species or sibling species which require a different way for identification. According to studies at the genic level, the mechanisms of species maintenance are not only reproductive isolation, but possibly a genetic consequence [13,14]. Stebbins stressed that species must be a group of populations discontinuous in morphology, and these discontinuities must have genetic bases [61].

We believe that species are preserving units of superior gene assemblies with distinct attributes and are maintained by certain mechanisms. This species concept includes three kinds of meaning. First, species is a preserving unit of superior gene assemblies, preservation of gene pools that the Biological species concept emphasized are actually an extreme form of species preservation. Second, a certain kind of gene assembly must have a unique aspect in morphology or physiology, and different gene assemblies have different morphological or physiological characteristics, such that species are thus recognizable. Third, preservation and maintenance of superior gene assemblies of species require a certain mechanism, but it is necessary to study the preservation mechanism before we elucidate speciation.

2 Species in plants and a blueprint for research

2.1 Species in plants

A few researchers doubt the reality of discrete species existence in plants, and believe that plant species are products of the subjective imagination of botanists [62,63], which are not objective and separate entities representing unique lineages or evolutionary units. Rieseberg *et al.* [64] designed two ways to test this hypothesis. First, they used statistical methods to analyze the corresponding relationships of phenetic clusters to species to test whether species are objective and discrete entities. Second, they used a hybrid index of post-fertilization isolation to test whether species or phenetic clusters are reproductively isolated lineages. Their phenetic analyses indicated that most taxa indeed have phenetic discontinuities, i.e. discrete clusters, 83 percent in plants but 88 percent in animals, but the proportion of species in these phenetic clusters is low, 52.8 percent in plants and 52.1 percent in animals. Lacking correspondence between species and phenetic clusters was attributable to over differentiation, clonal reproduction and polyploidization could have reduced the correspondence when taxonomy, life cycle, and hybridization had no impacts on the correspondence. That study implied that hybridization does not predominantly cause taxonomic problems, while reproductive isolation is a factor in the formation and maintenance of discrete morphological clusters. Taxa with strict reproductive isolation tend to have a distinct morphology. Rieseberg *et al.* [64] found that most plant species (phenetic clusters) indeed represent reproductively unique lineages, especially fern species correspond well to the notion of reproductively isolated lineages, while the correspondence in birds is lowest. In general, plant species have stronger isolation than animal species. This suggests that many plant species do reflect lineages of reproductive isolation, and thus, represent real biological entities. Because the hybrid index used in that study is based only on post-fertilization and reproductive isolation has mechanism more than post-fertilization isolation, as a result, the real proportion of plant species which represent lineages of reproductive isolation is higher. Clonal reproduction does cause problems for plant species, but the proportion is quite low in plants, less than 1%, only 126 of 13000 plant genera have asexual reproduction [64]. Consequently, plant species are indeed discrete and objective entities, and represent lineages of reproductive isolation. Based on many years of field experience and taxonomic practice, we think plant species are entities of objective existence.

Most higher plants are not diversified in the simple bifurcate manner of Hennig, but rather consist of a complicated reticulate pattern of evolution by hybridization and genetic introgression [65]. Reproductive isolation is not common between plant species. In addition, those plants with asexual reproduction which are not applicable to the Biological species concept have another special name, i.e. agamospecies (microspecies). Because issues concerning the Biological species concept exist, discussion concerning the species problem have not been suspended after the publication of Mayr's [20] Biological species concept [21,48]. Subsequent biologists have been trying to refine and provide a more reasonable concept.

2.1.1 The species concept in plant taxonomy and noticeable problems

For most plants, hybridization and genetic exchanges don't function as general criteria for establishing species. Jeffrey [66] modified the Biological species concept of Mayr, and developed an operable species concept in practice according to the complexity of plant variation. He believed that species are empirical units of classification and consist of a series of similar intergrading and interfertile populations, recognizably distinct from such series, and separated from other such series by genetically controlled barriers preventing interbreeding. Species are the same recognizably distinct and reproductively isolated series of intergrading and interfertile populations. Jeffery [66] used two key words in his species concept, i.e. recognizable distinctness and reproductively isolated. Jeffery's [66] species concept has difficulties in practice, the recognizable distinctness and reproductive isolation are not synchronous, thus, it may be possible that there are morphologically difficult but reproductively isolated cryptic species. Jones & Luchsinger [67] mentioned that although tetraploid and diploid individuals are not interfertile, they may be treated as a single species in taxonomic practice.

In taxonomic activities, delimitation of species is actually the application of the species concept in practice. From a biological viewpoint, species are existing forms to preserve superior gene assemblies, a certain gene assembly performs a certain unique set of morphological and physiological features. As a result, species are predictable by means of morphological and physiological characteristics. The leading definition of species in taxonomic practice is the Morphological species concept which is based upon morphological and geographical characteristics. In other words, the morphological species in plants are taxonomic entities based on temporal and spatial variation, such that the major points for this practical definition are discontinuity and correlation.

All volumes of the *Magnus Opus Flora Republicae Popularis Sinicae* have been published, including 80 volumes and 126 books, recording 31180 species of plants in China [68], with 16 volumes of the revised version, *Flora of China*, published. The status of quite a few species remain unclear in taxonomy. Currently several major problems are present in plant taxonomy. First, the available evidence or materials are not complete or are inadequate, knowledge of many species remains based on a few housed specimen or one single collection in many cases. Such examples are insufficient to provide a full picture of the variation between species, and don't clearly indicate the structure and distribution of populations. Second, many species complexes in taxonomy need full and exhaustive studies. As a result, not only do many species remain undescribed and unnamed, but many more have been described and named more than once. Different scientific names have been applied to what may eventually prove to be different individuals of the same species. The key to resolving these issues is to use appropriate analytical methods to analyze patterns of variation based on field observations and investigation.

2.2 Phases for plant species research

In order to describe new species it is not necessary to determine the preservation mechanisms of their superior gene assembly, Mayr, the leader of the Biological species concept, repeatedly stressed that species descriptions do not need to be based on experiments of reproductive isolation [69]. In most cases, description of new species is a type of deduction based on morphological and physiological characteristics. In the practice of plant taxonomy, a practical definition of species is often accepted as being the minimal taxonomic entities which have stable distinction and are identifiable in a common manner. This kind of deduction facilitates prediction to a certain extent, for the degree of predictability is the standard for a good or a poor taxonomy, such that high predictability suggests a good taxonomy while low predictability implies a poor taxonomy. Two factors which influence the predictability of taxonomy are the completeness of materials and the reliability of analyses. Currently, three major problems exist in plant taxonomy. First, many species have only one or two collections housed in herbaria, suggesting that the real variation for these species remains unclear. Predictability for this kind of classification is poor. It is often difficult to identify new materials intermediate between two species sharing a close resemblance, such that continuous variation in nature was frequently considered as disjunct variation because of inadequate sampling [23]. Second, many species complexes in taxonomy appeared because these species were based on inadequate materials and qualitative analyses and independent study of local researchers would bias the observation of character variation. Better predictability for these species might be based on observations of populations coupled with quantitative analyses of characteristics. Third, many species lack field observations and intensive collection. Without field investigation definitive characteristics might not be observed in a herbarium specimen. An example is the genus *Impatiens* which has fleshy leaves and shoots and tender floral parts. It is difficult to distinguish and identify the herbarium specimen if floral anatomy was not prepared during the field investigation. A described species may approach a good hypothesis when macromorphological variation is supported by palynological, chromosomal, and anatomical evidence. Answering the species problem can't be separated from the study of the mechanisms of speciation and species maintenance. Accordingly, we believe that plant species are clarifiable step by step.

In the first stage, discontinuous patterns of morphological variation are helpful for predicting most species. In taxonomic practice, we agree with Jeffery's [66] recognizability of species, namely species are minimal taxonomic entities that have stable distinctness and are recognizable or identifiable in a common way. Many species in plants are known to science from one single specimen or a very few collections. Others are solely known from flowers while yet others are only known from fruits. Inadequate materials are insufficient to provide a full picture of variation, population structure and distribution. The relationships of these species complexes remain ambiguous. Pertinent field investigation and intensive collection together with quantitative analyses of character variation may prove to be the key to resolving these vital issues. The first target of the Global Strategy for Plant Conservation (GSPC) is to document plant species [70]. The reliability of that checklist depends upon full observations of the variation of plants.

In the second stage, palynological, chromosomal, anatomical, and other available characteristics support the discontinuity of macromorphology, which could generate better species hypotheses. Those described species are hypotheses which need repeated testing by new data and new analytical tools, for good species identifications are amenable to new evidence. The more supportive the evidence is, the better the predictability is. DNA barcoding is a factor in testing these species hypotheses and would further cause researchers to restudy those described species to determine whether or not these species include sibling species or cryptic species.

In the third stage, mechanisms of speciation and species maintenance need study. This process is actually to clarify the preservation of any given superior gene assembly which has a key impact on the better understanding of species concepts.

3 Perspectives

The year 2009 was the 200th anniversary of the birth of Charles Darwin and the 150th anniversary of the publication of the book *Origin of Species*. Various international and domestic institutions organized a series of scientific activities to commemorate this naturalist who profoundly influenced human thought. The first issue of the *American Journal of Botany* in 2009 (vol 96 issue 1) is a special issue in memory of Darwin, which discusses the career and impact of Darwin. Both *Science* and *Nature* opened a new column to publish papers in memory of Darwin to discuss the key problems that Darwin discussed, e.g. speciation [71] and natural selection [72]. We believe that the most important question to discuss in memory of Darwin and his publication of *Origin of Species* is the species problem.

We hope that our analysis has made clear the crux of the species problem, yet the mechanisms of preservation and maintenance of superior gene assemblies remain unclear. As a result, the species problem merits extensive further consideration. The Modern evolutionary synthesis once pushed the discussion of species concepts into a frenzy around the 100th anniversary of the publication of *Origin of Species*. In the last half century, evidence from molecular biology, genetics, ecology, and conservation biology has rapidly accumulated. We expect a new synthesis of evolutionary biology around the 200th anniversary of the publication of Darwin's *Origin of Species* which may push the species problem and systematic biology to a higher level.

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