



Origin and evolution of species of *Hippophae* genus and formation mechanism of important traits



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5. Several interesting questions

1. Sea buckthorn is special species with multi purposes

1.1 Sea buckthorn is a pioneer species for ecological protection

sea buckthorn (*Hippophae* L.) is a pioneer species for soil and water conservation and ecological construction. There are more than 2.13 million ha of sea buckthorn in China, with more than 33.3 thousands ha newly planted each year.



1.2 Berry pulp producing guice and leaves producing tea

sea buckthorn berry pulp is rich in vitamin C and other biocomponents, widely used to produce juice, jam and other products; Sea buckthorn leaves is rich in flavonoids, widely used to make sea buckthorn tea.



1.3 Sea buckthorn oils with nutrient and medical values

Oils from sea buckthorn fruits is rich in omega-3, 6, 7, 9 fatty acids, flavonoids, V_E and other bioactive components, which is called as human edible oil in the 21st century.





2. Species of *Hippophae* genus and their origin and evolution



2.1 Species and subspecies of *Hippophae* genus

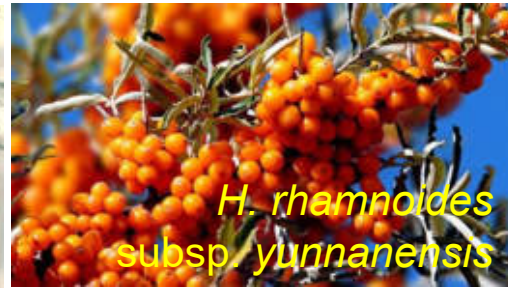
- There is six species and 12 subspecies.
- Seven species and 11 subspecies, in which *H. goniocarpa* subsp. *litangesis* Lian et X,L, Chen was described as a species.



H. salicifolia



H. rhamnoides
subsp. *sinensis*



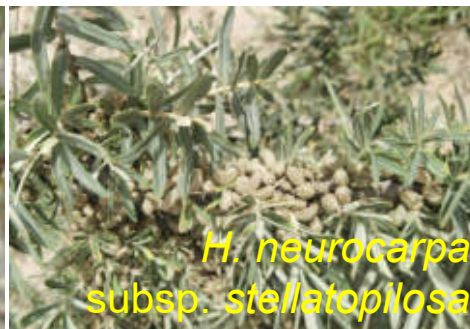
H. rhamnoides
subsp. *yunnanensis*



H. rhamnoides
subsp. *mongolica*



H. gyantsensis



H. neurocarpa
subsp. *stellatopilosa*



H. tibetana



H. goniocarpa
subsp. *goniocarpa*



H. goniocarpa
subsp. *litangesis*

H. salicifolia D. Don

H. rhamnoides

H. rhamnoides subsp. *sinensis* Rousi

H. rhamnoides subsp. *yunnanensis* Rousi

H. rhamnoides subsp. *turkestanica* Rousi

H. rhamnoides subsp. *mongolica* Rousi

H. rhamnoides subsp. *caucasia* Rousi

H. rhamnoides subsp. *carpatica* Rousi

H. rhamnoides subsp. *rhamnoides*

H. rhamnoides subsp. *fluviatilis* van Soest

H. gyantsensis (Rousi) Lian

H. neurocarpa

H. neurocarpa subsp. *stellatopilosa* Lian et X. L. Chen

H. neurocarpa subsp. *neurocarpa*

H. tibetana Schlecht

H. goniocarpa

H. goniocarpa subsp. *litangesis* Lian et X,L, Chen

H. goniocarpa subsp. *goniocarpa*



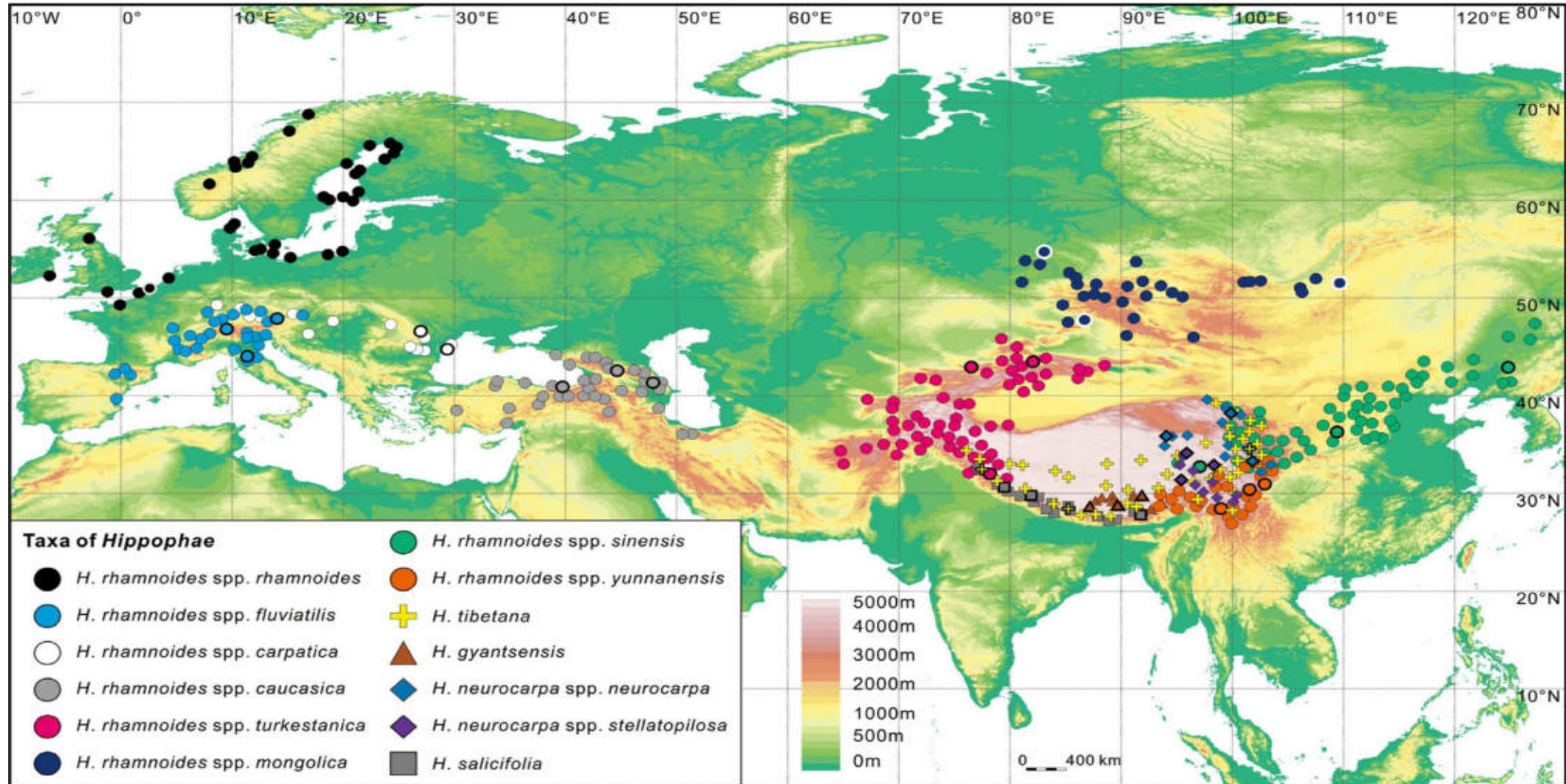
2. Species of *Hippophae* genus and their origin and evolution



2.2 Important traits of species of *Hippophae* genus

Species or subspecies	Interesting traits	Disadvantage traits
<i>H. salicifolia</i>	High Vc (1700mg/100g)	Low palmitoleic acid (C16:1, omega 7) of 1.1% in berry pulp
<i>H. rhamnoides</i>		
<i>H. rhamnoides</i> subsp. <i>sinensis</i>	Strong tolerance to drought, High Vc (100-1000mg/100g), high ω 7 of 31%	Small fruit and seeds, low oil content in seeds (7-10%), thorniness
<i>H. rhamnoides</i> subsp. <i>yunnanensis</i>	Strong tolerance to water logging, high ω 7 of 29%	
<i>H. rhamnoides</i> subsp. <i>mongolica</i>	Big fruit, high fruit yield, high oil contents in pulp and seeds compared with subsp. <i>sinensis</i>	Not tolerant to waterlogging; susceptible to dry shrinkage disease
<i>H. rhamnoides</i> subsp. <i>turkestanica</i>	Different eco-types with different tolerance to water stress	
<i>H. gyantsensis</i>	High oil content in seeds (22%), big fruits	Low palmitoleic acid of 0.54%; Vulnerable to insect pests
<i>H. neurocarpa</i>		
<i>H. neurocarpa</i> subsp. <i>stellatopilosa</i>	High oil content in seeds (16.6%)	few pulp, Low palmitoleic acid of 1.36%
<i>H. tibetana</i>	Big fruits, high oil content in pulp and seeds (36% and 18%), tolerance to high altitude and high radiation	No distribution in low altitude regions

2.3 Distribution of species of *Hippophae* genus in the world



(cited from Jia and Bartish, 2018)



2. Species of *Hippophae* genus and their origin and evolution



2.4 Formation of species from overlapping distribution area of different species in sea buckthorn

- *H. goniocarpa* is origin from crossing between *H. rhamnoides* ssp. *sinensis* and *H. neurocarpa* based on the same distribution regions, morphological traits, RAPD, cpDNA and cpDNA trnS-G analyses. It is origin of bidirectional hybridization, but the main maternal source should be Chinese seabuckthorn.



- *H. goniocarpa* subsp. *litangensis* Lian et X,L, Chen is origin from crossing between *H. rhamnoides* ssp. *yunnanensis* and *H. neurocarpa* ;
- *Hippophae gyantsensis* is origin from crossing between *H. neurocarpa* and *H. rhamnoides* ssp. *yunnanensis*.





2. Species of *Hippophae* genus and their origin and evolution



➤ Two interesting populations

① Langxian seabuckthorn, which is growing in Lanxian county of Lingzhi city in Tibet.



② Guoluo seabuckthorn, which is growing in Guore village of Zuogng county of Changdu city in Tibet.





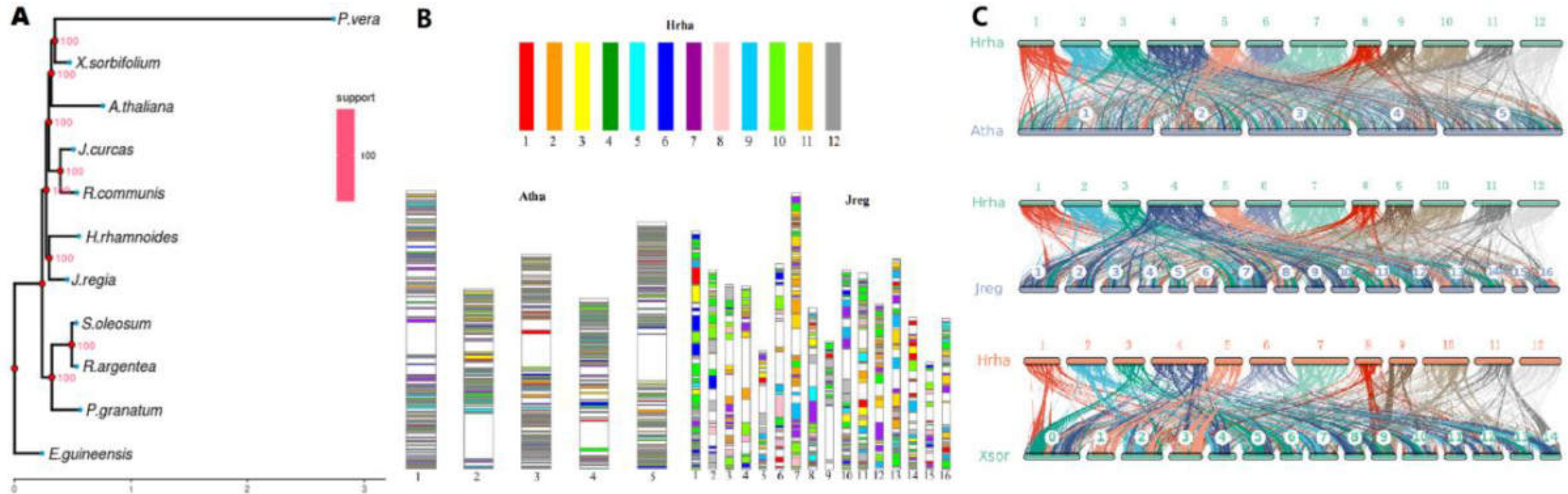
2. Species of *Hippophae* genus and their origin and evolution



2.5 Origin and evolution of species of *Hippophae* genus

2.5.1 Constructed whole genome maps of three sea buckthorn species/subspecies

(1) The genome size of the excellent seabuckthorn variety 'Shiyou 1' (*H. rhamnoides* subsp. *mongolica*) is 1.27G, with a repeat sequence proportion of 72.35%. A total of 26429 coding genes were predicted (99.8% of genes could predict function), and 98.32% of genes were anchored on 12 chromosomes.



‘实优1号’基因组系统进化 (A)、染色体重排 (B) 和共线性 (C) 分析结果



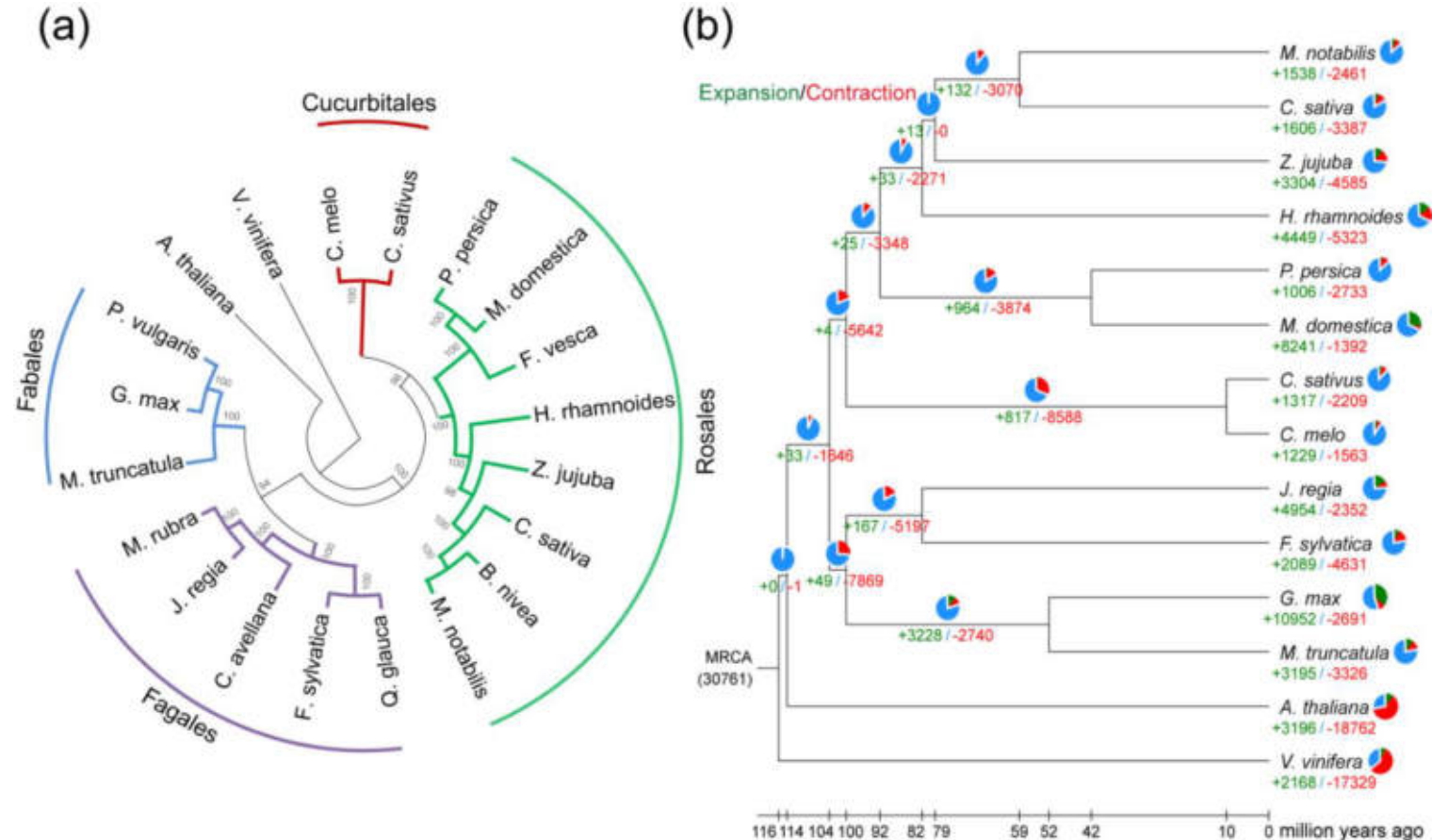
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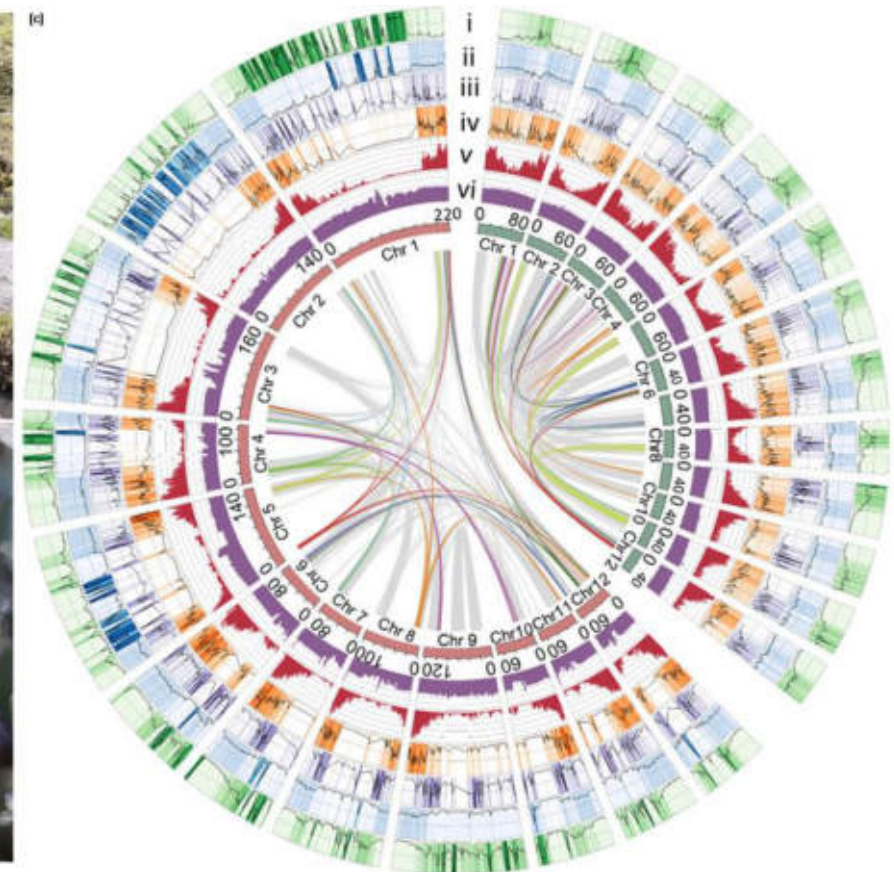
(2) The genome size of *Hippophae rhamnoides* spp. *sinensis* is 730Mb, scaffold N50: 65 Mb, 30812 protein encoding genes were predicted to be distributed on 12 chromosomes; The relationship between *Hippophae rhamnoides* spp. *sinensis* and jujube (*Ziziphus jujuba*) is relatively close.



2.5 Origin and evolution of species of *Hippophae* genus

2.5.1 Constructed whole genome maps of three sea buckthorn species/subspecies

(3) The genome size of *Hippophae tibetana* is 1452.75 Mb, with 33367 encoding genes and Contig N50 of 74.31 Mb; Compared with its relative species, it has undergone rapid gene evolution to adapt to high altitude habitats.





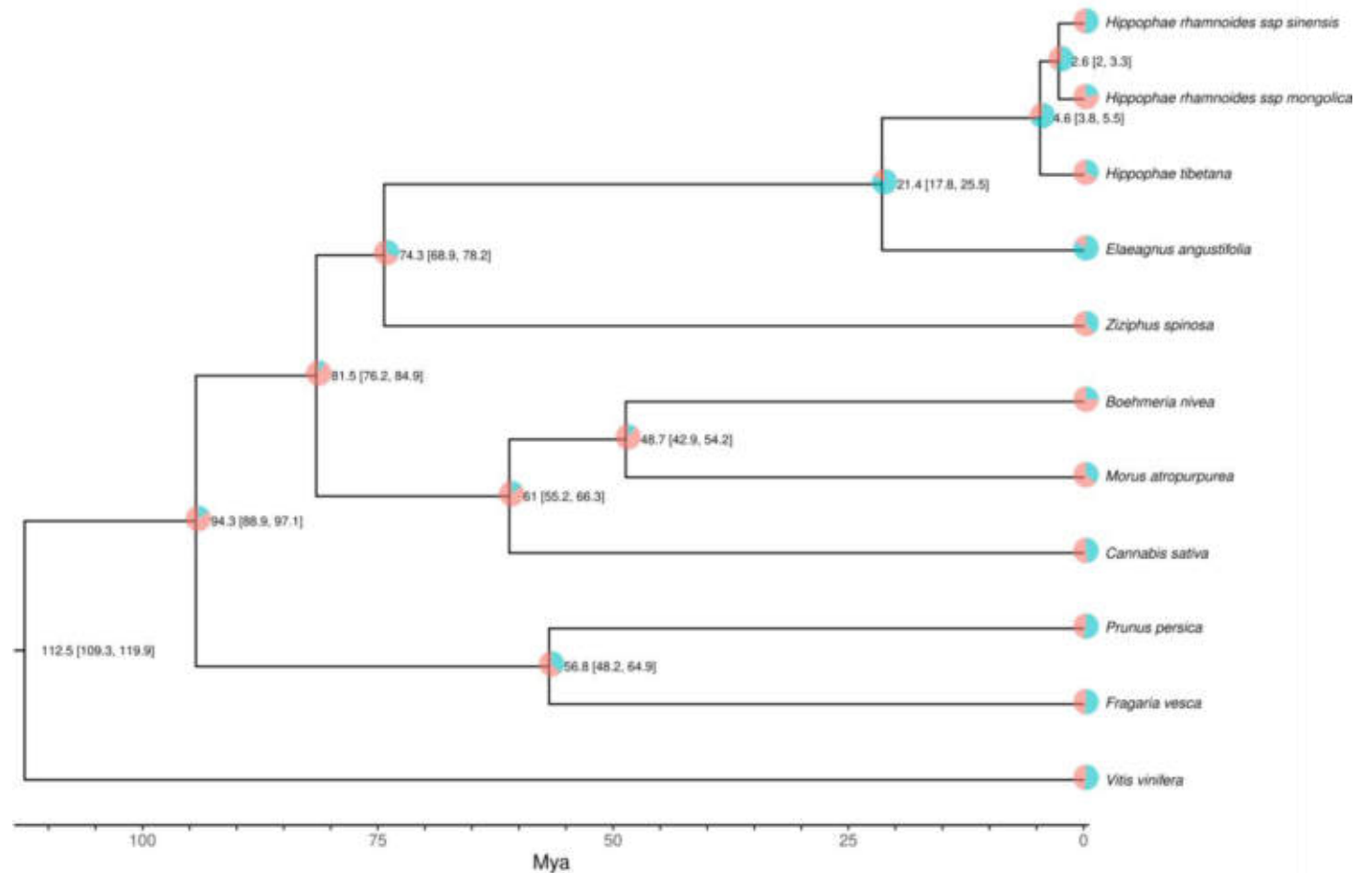
2. Species of *Hippophae* genus and their origin and evolution



2.5 Origin and evolution of species of *Hippophae* genus

2.5.2 Pangenome of three sea buckthorn species/subspecies

- ① The divergence time of three seabuckthorn (*H. rhamnoides* ssp. *mongolica*, *H. rhamnoides* ssp. *sinensis*, *H. tibetana*) and *Elaeagnus angustifolia* was ~21.4 million years ago. The divergence time between them was ~4.6 million years ago, while the divergence time between ssp. *sinensis* and ssp. *mongolica* was ~2.6 million years ago.
- ② There are significant differences in the number of gene families involved in the expansion, contraction, and rapid evolution of the three sea buckthorn species, indicating significant differences in their differentiation process.



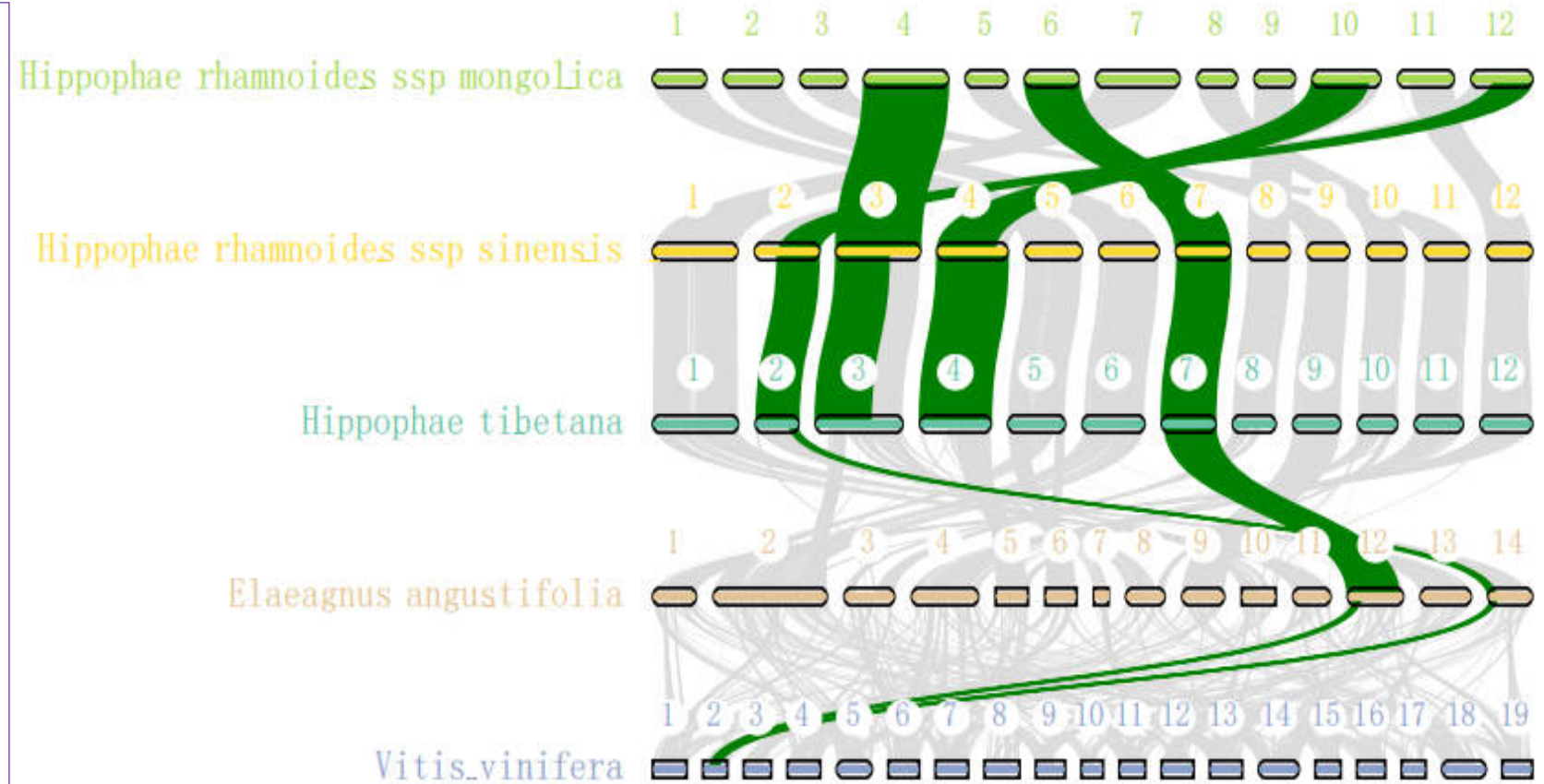
Evolutionary trees and divergence time of 11 species

Note: The number differentiation time and 95% CI at the node are shown in the pie chart, which represents the proportion of gene families undergoing expansion (green) and contraction (red)

2.5 Origin and evolution of species of *Hippophae* genus

2.5.2 Pangenome of three sea buckthorn species/subspecies

- ③ Through collinearity analysis of three sea buckthorn species with their closely related species of *Elaeagnus angustifolia* and *vitis vinifera*, it was found that there is a high degree of collinearity among the three sea buckthorn species, indicating a high degree of conservation among them. Compared with the closely related species *Elaeagnus angustifolia* and grape, the three seabuckthorn species showed significant differentiation and exhibited obvious doubling events.



Collinearity analysis of three sea buckthorn species with their closely related species of *Elaeagnus angustifolia* and *vitis vinifera*



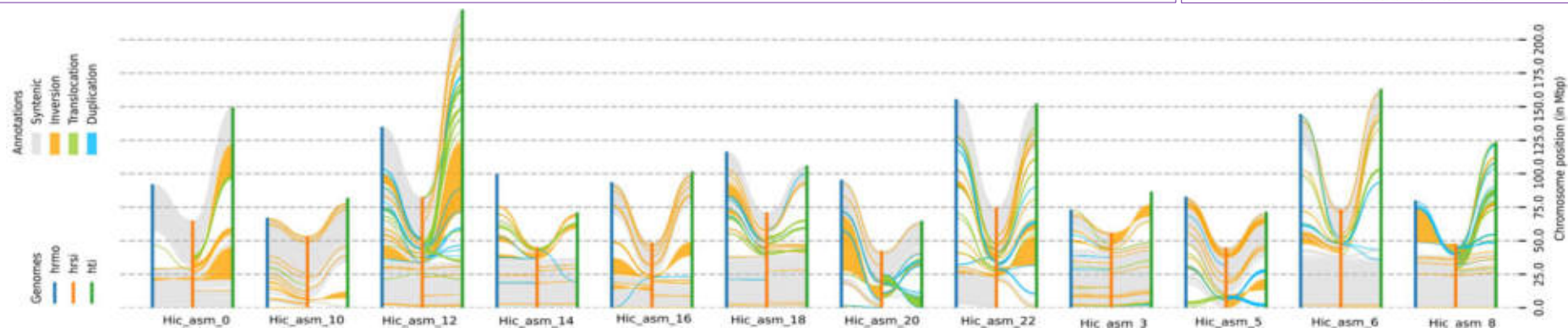
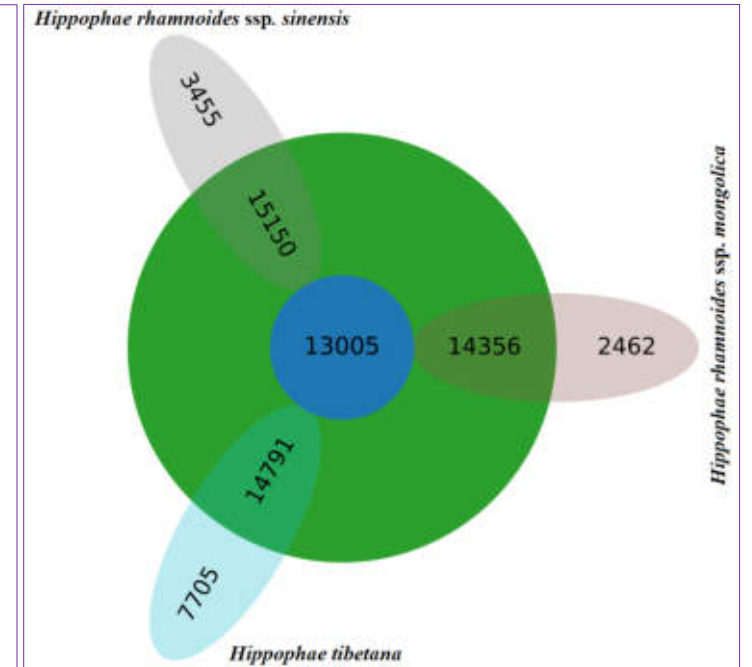
2. Species of *Hippophae* genus and their origin and evolution



2.5 Origin and evolution of species of *Hippophae* genus

2.5.2 Pangenome of three sea buckthorn species/subspecies

- ④ There are 13005 core genes sharing in three seabuckthorn species, 15150 sub core genes in the ratio of spp. *sinensis* to spp. mongolian and *H. tibetana*, 14356 sub core genes in the ratio of spp. mongolian to spp. *sinensis* and *H. tibetana*, and 14791 sub core genes in the ratio of *H. tibetana* to spp. mongolian and spp. *sinensis*. There are 3455 private genes of spp. *sinensis*, 2462 private genes of spp. mongolian and 7705 private genes of *H. tibetana*.
- ⑤ The genome alignment of three genomes showed a collinearity of 613Mb. In addition, small local genomic variations were found between the three genomes, including 4503159 SNPs. In addition, it can be seen that besides the collinearity region, there are also abundant inversions, and there are many large fragment inversions.





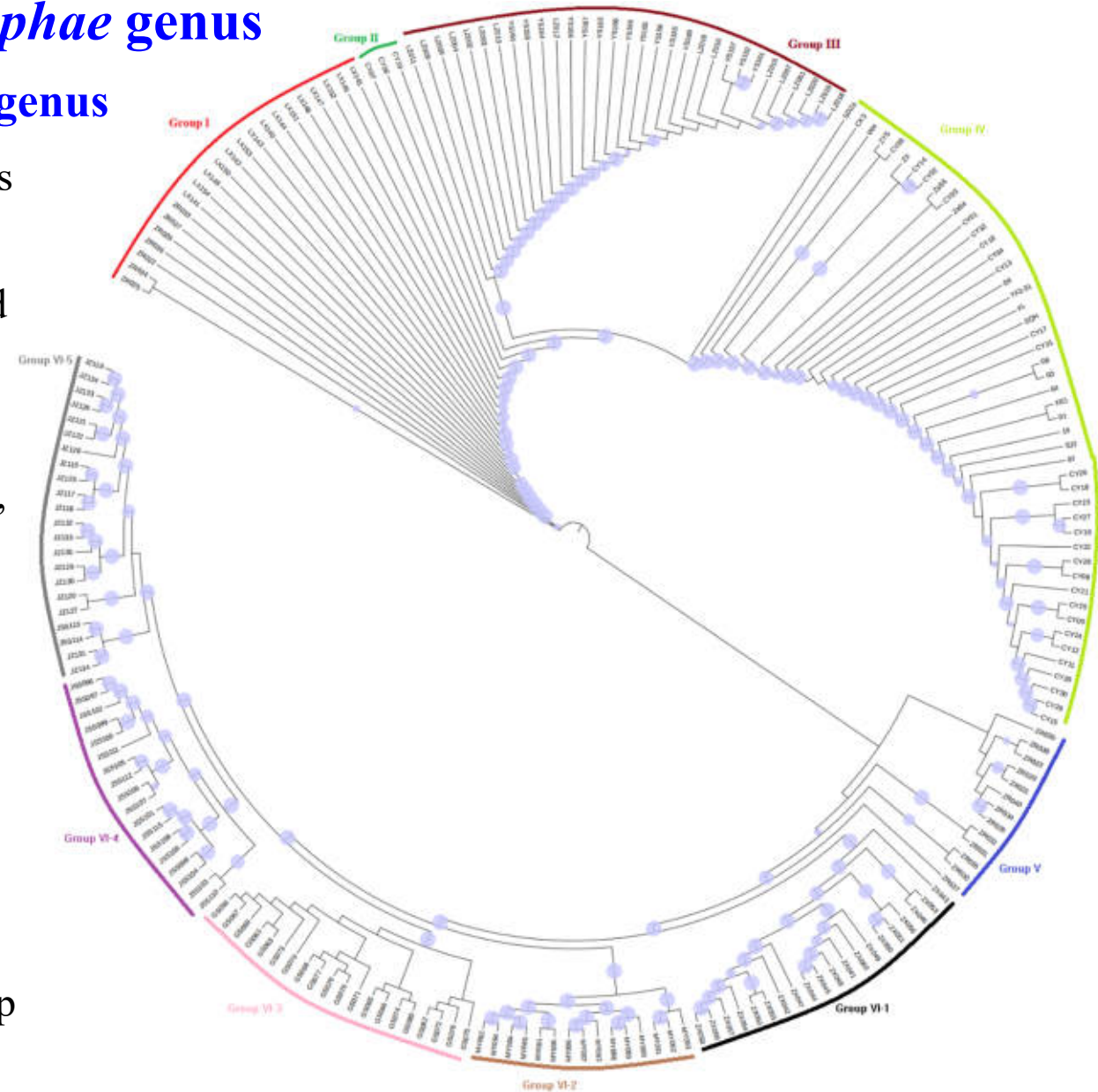
2. Species of *Hippophae* genus and their origin and evolution



2.5 Origin and evolution of species of *Hippophae* genus

2.5.3 Evolution relationship of species of *Hippophae* genus

- 212 materials were clustered into 6 groups, with 20 samples from *H. salicifolia* growing in the Niyang River Basin of Zhare Village and 15 materials from Lang County clustered in Group I.
- Three spp. *sinensis* samples from Chaoyang were clustered in Group II, which is located in the middle of *H. salicifolia*, ssp. *yunnanensis*, and spp. *mongolian*, reflecting the position of spp. *sinensis* in the evolution of *Hippophae* genus.
- The 16 sea buckthorn materials from Linzhi Town and 15 sea buckthorn materials from Yusong were clustered in Group III. ssp. *yunnanensis* materials from Yusong Village in Milin County and Linzhi Town did not show significant geographical isolation because the samples within the group intersected with each other.



2.5 Origin and evolution of species of *Hippophae* genus

2.5.4 Gene flow between different seabuckthorn populations

- Based on gene flow analysis, *H. salicifolia* is an older species, which later evolved into ssp. *yunnanensis* in one direction, and *H. gyantsensis* in the other direction. However, due to geographical isolation and other reasons in the evolution process, the ZXC population on the Niyang River (ZRL, ZRC) and the Lasa River on both sides of the Miraj Mountains is closer, while the four populations (GSC, MYC, JSS, and JZX) on the the Yarlung Zangbo River tributaries are closer. In this branch, GSC population has greater geographical isolation, and the evolutionary relationship between MYC, JSS, and JZX populations is closer.
- There is a certain gene flow between *H. gyantsensis* and Langxian's seabuckthorn, which can explain why the seabuckthorn fruit in Langxian is significantly different from that in other regions. However, the name and definition of the Langxian's seabuckthorn still need further verification.

