Biological Diversity

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Common but Least Noticed: Diversity of the Mangrove Fern *Acrostichum aureum* in Hainan Island, China

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ABSTRACT

Acrostichum aureum across nine coastal sites in Hainan Island was examined using morphological, cytometric, and genomic approaches. Cluster analysis identified five ecologically distinct clades linked to pinna morphology and habitat. Flow cytometry suggested sexual reproduction without ploidy-spore number correlation. Genetic analyses revealed cohesive population structure with minor introgression from *Acrostichum speciosum* (12 samples), relatively low nucleotide diversity (π = 0.0647–0.0780), outcrossing dominance indicated by high heterozygosity (*Ho* = 0.0771–0.1042) and negative inbreeding coefficients (*Fis* = −0.0407–0.0017), and minimal differentiation (*Fst* < 0.05) among populations. Despite widespread distribution, the species exhibits moderate genetic variation, highlighting vulnerability to environmental changes. Based on the heterozygosity and π, three priority conservation areas are proposed to preserve extant genetic diversity and maintain habitat connectivity. These findings underscore the need for targeted protection of this ecologically significant mangrove fern.

1 | Introduction

Mangroves are woody halophyte-dominated wetland plant communities primarily found in tropical and subtropical coastal intertidal zones. These ecosystems thrive in extreme transitional habitats between marine and terrestrial environments [1], providing critical ecological services such as coastal protection against storms, tidal surges, and tsunamis, as well as supplying resources for local communities [2]. However, mangroves face severe degradation due to anthropogenic activities, with over half of the world's original mangrove cover already lost or degraded [1, 3].

In China, mangroves are concentrated in Hainan, Guangdong, Guangxi, Fujian and Taiwan. Hainan Island, situated at the northern edge of global mangrove distribution, hosts 32 true mangrove species (including 6 exotic) and 31 semi-mangrove species (including 2 exotic) [4]. Despite its ecological significance as a mangrove biodiversity hotspot, Hainan's mangrove area has declined dramatically—from nearly 10,000 ha in the

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1950s to 4891 ha by 2013 [5–8]. Genetic studies reveal alarmingly low diversity in key species (*Ceriops tagal*, *Sonneratia ovata*, *Rhizophora apiculata*, and *Avicennia marina*), rendering them vulnerable to extinction under climate change and human pressures [9–12]. Small effective population sizes and weak adaptive capacity further exacerbate these risks [13]. Therefore, urgent conservation measures are needed to safeguard Hainan's native mangroves, focusing on maintaining population sizes and genetic diversity to ensure long-term resilience [14].

Acrostichum aureum (Pteridaceae) is a vital component of coastal mangrove ecosystems, distributed across pantropical regions, including Hainan, Guangdong, Guangxi, Taiwan, Hong Kong, and Macao. As the first line of defense for mangroves, it serves as a sensitive bioindicator of environmental changes [15–18]. Despite its ecological importance, *A. aureum* has received less research attention compared to dominant mangrove taxa such as *Sonneratia*, *Avicennia*, *Ceriops*, *Rhizophora,* and *Bruguiera* [10, 19–23]. Existing studies primarily focus on its pharmacognosy, chemical constituents, and biological activities [24–30], yet its genetic diversity remains poorly understood. Investigating *A. aureum*'s genetic diversity in Hainan Island is critical for mangrove ecosystem stability and biodiversity conservation.

Genetic diversity, narrowly defined as intra-species gene variation or broadly as the totality of genetic information in ecosystems [31], determines a species' adaptive potential. Populations, the fundamental units of evolution, exhibit distinct distribution patterns, making genetic diversity a measure of both variation and population genetic structure. Advances in molecular biology, including polygene association analysis, transcriptome sequencing, reduced-representation genomics and whole-genome sequencing, now enable comprehensive assessments of genetic diversity and phylogenetic relationships in mangroves [10, 13, 19–23, 32–35].

Given the ecological importance of mangroves and the severe degradation of Hainan's mangrove ecosystems, this study takes *A. aureum* in Hainan Island as a representative example and examines its genetic structure and variation to assess its genetic diversity levels, identify diversity hotspots and vulnerable populations, and establish scientific criteria for prioritizing conservation areas. By addressing these gaps, we aim to advance mangrove protection strategies through evidence-based genetic insights.

2 | Materials and Methods

2.1 | Sample Collection, Correlation Analysis of Morphological Characteristics and Habitat Types

A total of 122 *A. aureum* samples were collected from 12 populations in 9 cities and counties of Hainan Island (Figure 1), of which 68 samples contained fertile fronds (Supporting Information S2: Table S1). The pinna types were classified according to the morphology of the apex of the pinnae. Ten mature, full, uncracked and uncontaminated sporangia were randomly selected from the fertile fronds of *A. aureum* samples, and the number of spores in each sporangium was counted under the optical microscope. Based on the morphological data and habitat types, cluster analysis was performed with SPSS software on all *A. aureum* samples selected for genotyping-by-sequencing (GBS). Euclidean distance 15 was set as the classification criteria to analyze the correlation between the morphological characteristics of *A. aureum* and habitat types.

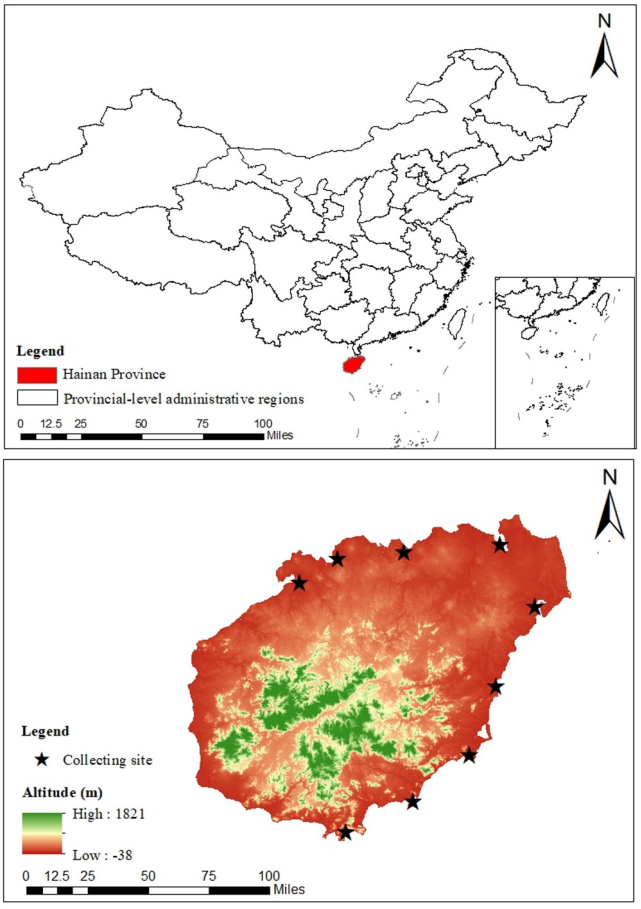


FIGURE 1 | Collecting sites of *Acrostichum* L in Hainan Island, China (The drawing review number is GS (2024) 0650).

2.2 | Chromosome Ploidy Analysis

In order to determine chromosome ploidy of gametophytes and sporophytes, 14 representative *A. aureum* samples were selected for seedling culture with their spores. The cultivation method was referred to [36].

A live *A. aureum* plant was selected as the reference sample for the determination of chromosome ploidy. The chromosome counting of the reference sample was performed following the protocol described by Zhang et al. [37]. The chromosome ploidy of gametophytes and sporophytes of other samples was determined by flow cytometry (German CyFlow Ploidy Analyser) with the method established by Tian et al. [38].

2.3 | Genotyping-By-Sequencing and Population Genetic Analysis

2.3.1 | Genotyping-By-Sequencing

Except for sample LS24, 121 *A. aureum* samples, with 8 *Acrostichum speciosum* samples and 1 suspected hybrid sample as the outgroup, were selected. Thus, a total of 130 samples were sent to the Novogene company (Beijing, China) for genotyping-by-sequencing (GBS) (Supporting Information S2: Table S1). First, DNA samples were detected, GBS library was constructed, and the constructed GBS library was checked, and then Illumina HiSeq PE150 sequencing was performed after the library test was qualified. After sequencing, Raw Data (original sequencing sequence) were obtained, and after removing interference information in the Data, Clean Data (valid data) were obtained. Then, the number of reads captured by MseI-+EcorI at both ends of clean reads and the ratio of captured Reads to the number of clean reads (enzyme capture rate) were counted to evaluate the enzyme digestion efficiency. Finally, Stacks are used for analysis without reference genomes. Clean reads were then assembled in Stacks v2.2. The consensus sequences (loci) were obtained by assembling (clustering) individual samples using ustacks (parameter: -M 4). The loci of all samples were aggregated into a catalog using cstacks (parameter: -n 4). Assembly, comparison and variation detection were performed using gstacks to obtain the number of loci and average sample coverage depth. After population filtering (parameter: -p 1 -r 0.80 --vcf --min_maf 0.01), single nucleotide polymorphisms (SNPs) of high quality were obtained and saved in vcf format.

2.3.2 | Population Genetic Structure Analysis

TreeBest (http://treesoft.sourceforge.net/treebest.shtml) was used to calculate the distance matrix. Then the Neighbor-Joining method (NJ) was applied to construct a phylogenetic tree. The bootstrap values were obtained after up to 1000 calculations. Finally, FigTree v1.4.4 was used to visualize the phylogenetic tree. Principal component analysis (PCA) was performed by GCTA (http://cnsgenomics.com/software/gcta/pca.html), and the result was mapped via the ggplot2 packages in R. In addition, PLINK input files (Ped files) (http://pngu.mgh.harvard.edu/~purcell/plink/) were created, and frappe software was used to build population genetic structure and construct population hereditary information.

2.3.3 | Genetic Diversity Analysis

Based on the filtered SNPs, the observed heterozygosity (*Ho*) and expected heterozygosity (*He*) at the population level, nucleotide diversity (π), inbreeding coefficient (*F*is) within populations, and genetic differentiation coefficient (*F*st) between populations were calculated by the Populations subprogram in Stacks v2.2.

3 | Results

3.1 | Correlation Between Morphological Characteristics of *A. aureum* and Habitat Types in Hainan Island

3.1.1 | Habitat Types

Based on ref. [39], field surveys identified six distinct habitat types for *A. aureum* populations, which were categorised along a moisture gradient (Figure 2). They were riverside (RS), lakeside (LS), swamp (SW), understory (UN), wasteland (WL), and shrimp pond edge (SP). Because of the strong human interference near the shrimp pond, SP exhibited similar edaphic conditions to WL. All six types of habitats are near the estuary.

3.1.2 | Pinna Types

The pinnae of *A. aureum* were categorized into six distinct types based on apex morphology (Figure 3), as follows: Type A, concave apex lacking a cusp; Type B, concave apex with a tiny cusp; Type C, truncated or blunt apex featuring a tiny cusp;

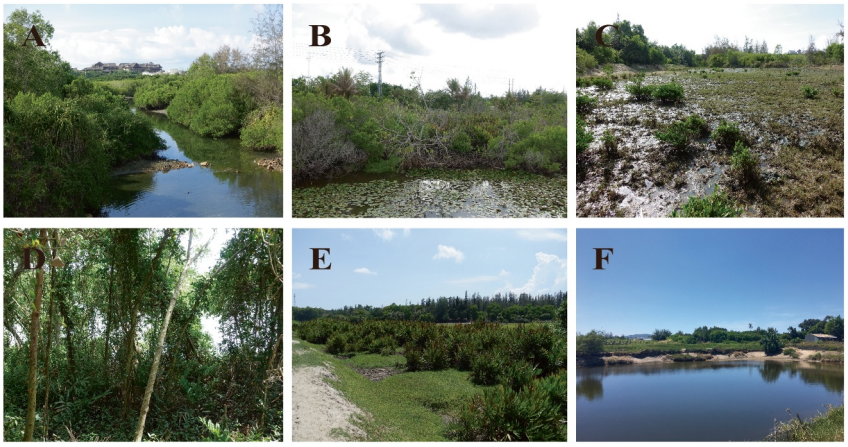


FIGURE 2 | Habitats of *Acrostichum aureum*. (A) Riverside - Sanya Yalong Bay Mangrove Forest; (B) Lakeside - Wenchang Bamen Bay Mangrove Forest; (C) Swamp - Sanya Yalong Bay Mangrove Forest; (D) Understory - Qionghai Shamei Village Wetland Park; (E) Wasteland - Shanniu Port, Li'an Town, Lingshui; (F) Shrimp pond edge - Dahua Village, Wanning Shenzhou Peninsula.

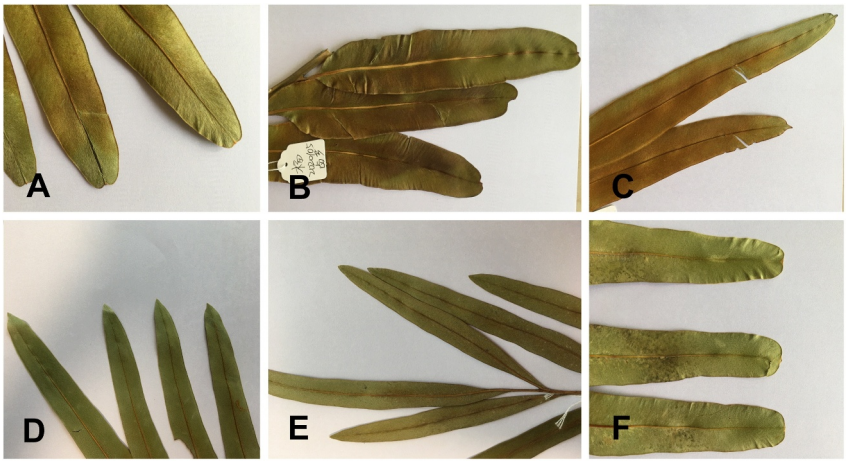


FIGURE 3 | Pinna types of *Acrostichum aureum*. (A) Type A, concave apex lacking a cusp; (B) Type B, concave apex with a tiny cusp; (C) Type C, truncated or blunt apex featuring a tiny cusp; (D) Type D, acuminate apex without a cusp; (E) Type E, sharply acuminate apex without a cusp; (F) Type F, rounded and blunt apex without a cusp.

Type D, acuminate apex without a cusp; Type E, sharply acuminate apex without a cusp; Type F, rounded and blunt apex without a cusp. Additionally, some specimens exhibited compound pinna types, displaying two or more of the above morphologies.

3.1.3 | Number of Spores

The sporangia of *A. aureum* contained 23–58 spores each. Based on the average spore count per sporangium, the samples were classified into four grades: Grade I, the average number of spores per sporangium was 20–29; Grade II, the average number of spores per sporangium was 30–39; Grade III, the average number of spores per sporangium was 40–49; Grade IV, the average number of spores per sporangium was 50–58. Grade II and III were the predominant spore quantity grades of *A. aureum* (Supporting Information S2: Table S1).

3.1.4 | Cluster Analysis

With pinna type, spore number and habitat type as parameters (Supporting Information S2: Table S1) and Euclidean distance 15 as classification criteria, cluster analysis was performed on all *A. aureum* samples selected for GBS. As shown in Figure 4, *A. aureum* in Hainan Island can be divided into 5 clades. Clade I consisted of 15 samples, from wasteland and the edge of shrimp ponds, with compound pinna types and the spore quantity grade III as the main morphological characteristics. Clade II contained 21 samples, from riverside, swamps, and lakeside. Their pinna types were mainly B and C, and their spore quantity grades were mainly II and III. Clade III (18 samples) shared identical habitat preferences and spore production patterns with Clade II. However, this group was distinguished by its strong predominance of compound pinna types. Clade IV consisted of 30 samples, primarily from riverside, mainly with compound pinna types and pinna type C. Only 1/8 samples had fertile fronds in Clade IV, and the spore quantity grade was grade I. Clade V contained the largest number of samples (37), primarily from the understory of mangroves and wasteland, and was dominated by compound pinna types and pinna type C. Only 1/4 of the samples in Clade V had fertile fronds, and the spore quantity grade was I and II. It is evident that there is a significant correlation between the pinna types of *A. aureum* and the habitat types. Wet habitats, such as riverside, swamp and lakeside, are dominated by *A. aureum* individuals with compound pinna types, pinna types B and C, while arid habitats, such as wasteland and shrimp pond edge, are dominated by individuals with compound pinna types. In summary, the spore quantity of *A. aureum* is mainly grade III and II, but there is no correlation between the number of spores and habitats or pinna types.

3.2 | Chromosome Ploidy and Reproductive Patterns of *A. aureum* in Hainan Island

3.2.1 | Flow Cytometry Results

Chromosome counting revealed that the root cells of the reference sample contained 2n = 60 chromosomes (Supporting Information S1: Figure S1A). This finding, consistent with the established base chromosome number of $x = 30$ for *A. aureum* [40], confirms the diploid nature of the reference specimen. A total of 17 samples comprising 6 sporophytes and 11 gametophytes were examined. The results demonstrated that the average peak value of sporophyte samples was similar to that of reference samples (Table 1), so these samples and reference samples were diploid. For gametophyte samples, the average peak value was half that of the reference sample, so the gametophytes of these samples were haploid.

3.2.2 | Appearance of Antheridia and Archegonia

Female and male gametophytes were clearly distinguished after 30 days post sowing. Spherical antheridia were near the root of the male gametophyte (Supporting Information S1: Figure S2B). Female gametophytes were larger than male gametophytes (Supporting Information S1: Figure S2A). The archegonium was

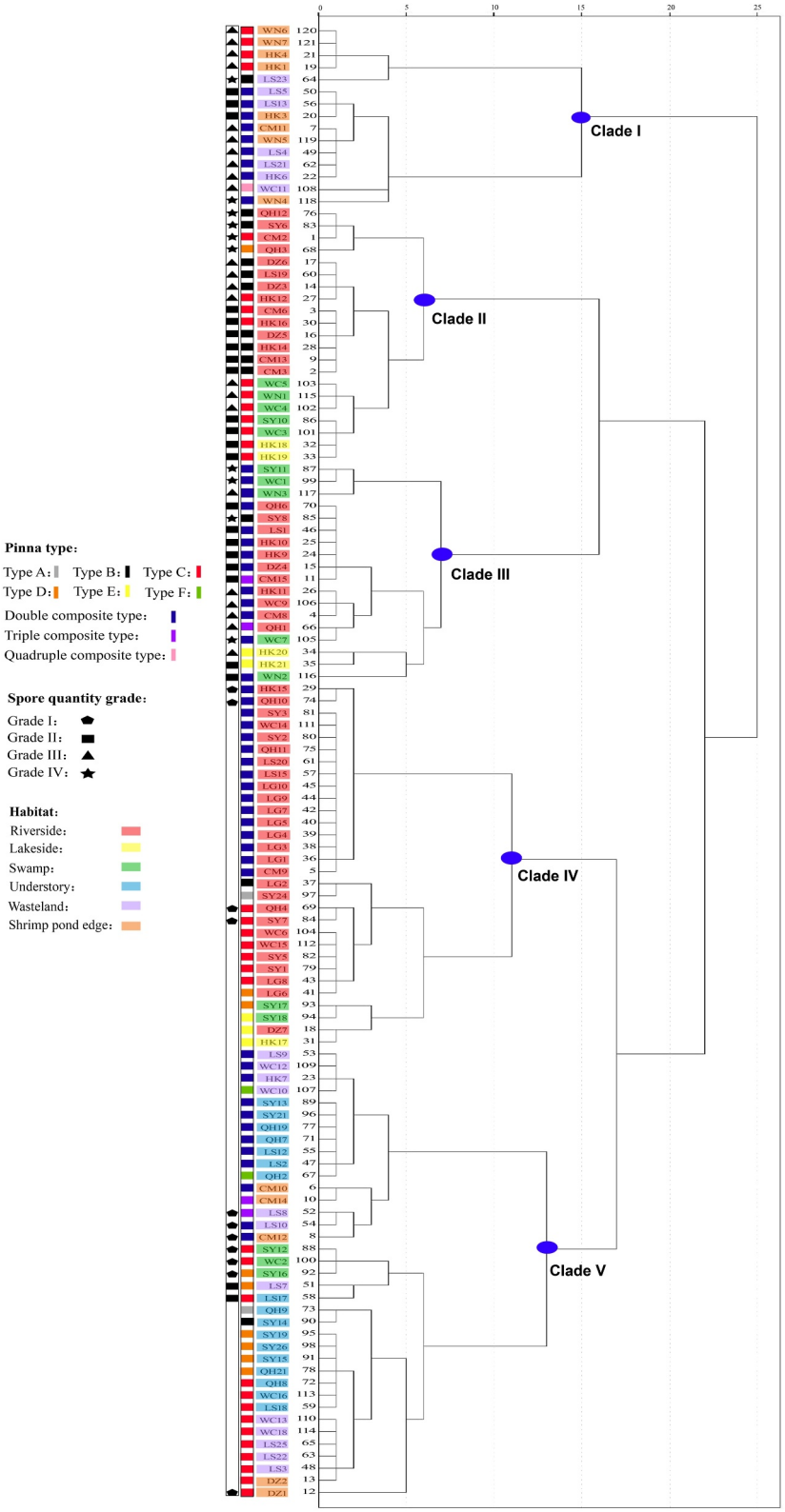


FIGURE 4 | Morphological clustering analysis of *Acrostichum aureum* in Hainan Island.

mainly born near the growth point on the ventral surface of the female gametophyte (Supporting Information S1: Figure S2C). When mature, the top of the archegonium was cracked, and four cells could be clearly seen on the top of the archegonium (Supporting Information S1: Figure S2D). Among the 14 *A. aureum* samples sown, HK21, LS24, LS8, LS23, and WN4 were all observed with antheridia and archegonia, but only antheridia were observed in the remaining samples, and no archegonia were found, which might be related to the various mature rates of female gametophytes.

TABLE 1 | Flow cytometry results of *Acrostichum aureum* in Hainan Island.

No.	Sample no.	Material type	Spore quantity grade	Parameter	Coefficient of variation	Median fluorescence intensity	Average value	Ploidy
1	CK	Y20211112-4 sporophyte	—	DNA	3.48	13031	13028.6	Diploid
2	HK21	Y2020126 sporophyte	II	DNA	2.99	12812	12793.9	Diploid
3	HK21*	Y2020126 gametophyte	II	DNA	4.94	7157	7146.63	Haploid
4	LS24	Y2020074 sporophyte	III	DNA	3.89	12723	12756.7	Diploid
5	LS24*	Y2020074 gametophyte	III	DNA	4.92	6493	6464.31	Haploid
6	LS8*	Y2020058 gametophyte	I	DNA	4.33	7264.5	7256.06	Haploid
7	LS23*	Y2020073 gametophyte	IV	DNA	4.05	6714	6694.91	Haploid
8	WN4*	Y2020079 gametophyte	IV	DNA	4.22	7085	7057.86	Haploid
9	HK10	Y2020115 gametophyte	II	DNA	4.44	6902	6869.09	Haploid
10	HK18	Y2020123 sporophyte	II	DNA	4.55	11459.5	11446.1	Diploid
11	HK19	Y2020124 sporophyte	II	DNA	4.06	12774	12739.4	Diploid
12	QH6	Y2020088 gametophyte	II	DNA	4.12	7355	7355.83	Haploid
13	WC9	Y2020103 gametophyte	III	DNA	4.08	7067	7048.09	Haploid
14	HK20	Y2020125 sporophyte	III	DNA	4.28	11840	11843.1	Diploid
15	WN1	Y2020076 gametophyte	III	DNA	4.32	6804	6780.99	Haploid
16	WN5	Y2020080 gametophyte	III	DNA	4.63	7200.5	7183.5	Haploid
17	WN6	Y2020081 gametophyte	III	DNA	3.99	7297	7293.09	Haploid

Note: * indicates the presence of archegonia.

3.2.3 | Inference of Reproductive Mode

Chromosomal analysis and gametangia observations provided conclusive evidence of sexual reproduction in *A. aureum*. The diploid sporophytes of samples HK21 and LS24 produced haploid gametophytes through meiosis, completing the first half of the sexual life cycle. This transition was further supported by the presence of both archegonia and antheridia on their gametophytes, confirming their bisexual nature and capacity for fertilization. Similarly, samples LS8, LS23, and WN4 exhibited haploid gametophytes bearing functional reproductive structures, indicating their potential to generate diploid sporophytes through syngamy. Notably, these sexually reproducing samples displayed a broad range of spore production (Grades I-IV), demonstrating that sexual reproduction persists across varying spore numbers.

3.3 | Population Genetic Characteristics of *A. aureum* in Hainan Island

3.3.1 | SNP Mining

Because most of the DNA bands were clear and bright (Supporting Information S1: Figure S3), all samples sent for testing met the quality requirements of sequencing. The total sequencing data volume of the tested samples was 313.09 Gb,

with an average of 2.41 Gb per sample. The amount of Clean Data after sequencing data filtering was 291.17 Gb, with an average of 2.24 Gb per sample. The results of sequencing quality distribution (Supporting Information S2: Table S2) showed that Q20 and Q30 of all samples were ≥ 91.19% and 79.55% respectively. The average base error rate ranges from 0.02% to 0.05%. GC content was 37.65%–39.61%, GC distribution was normal, and no samples were contaminated. The effective data rate ranges from 91.19% to 99.88%. The above quality evaluation results showed that the sequencing quality was high and the sequencing was successful. According to sequence similarity, the Clean Data of each individual was clustered and SNP_S were detected, and a total of 8066 SNPs were obtained.

3.3.2 | Population Genetic Structure

Phylogenetic analysis (Figure 5A) revealed that all *A. aureum* samples segregated into 12 distinct branches, while *Acrostichum speciosum* and the suspected hybrid (HK23) formed a separate outgroup clade, clearly distinct from *A. aureum*. This clear divergence supports the high reliability of the phylogenetic reconstruction.

Principal component analysis (PCA) (Figure 5B) further confirmed these findings, with all *A. aureum* samples clustering together, while the outgroup samples separated into two distinct groups. Notably, the suspected hybrid (HK23) grouped with *A. speciosum*, reinforcing its hybrid status.

To explore genetic relationships further, a population structure analysis was performed (Figure 5C). At the optimal K value of 2 (Supporting Information S1: Figure S4), the samples divided into two subgroups: Subgroup 1 (blue) comprised eight *A. speciosum* samples (WC19, WC20, WC23, WC25, WC27, WC29, WC30, and HK22), while Subgroup 2 (green) included all *A. aureum* samples along with the suspected hybrid (HK23). Interestingly, 12 *A. aureum* samples (CM9, CM10, CM11, CM12, CM14, CM15, DZ6, DZ7, SY1, SY2, SY3, and SY5) exhibited a minor genetic contribution from Subgroup 1, suggesting limited historical gene flow between *A. aureum* and *A. speciosum*. Strikingly, the suspected hybrid (HK23) displayed nearly equal ancestry proportions from both subgroups (about 50% each),

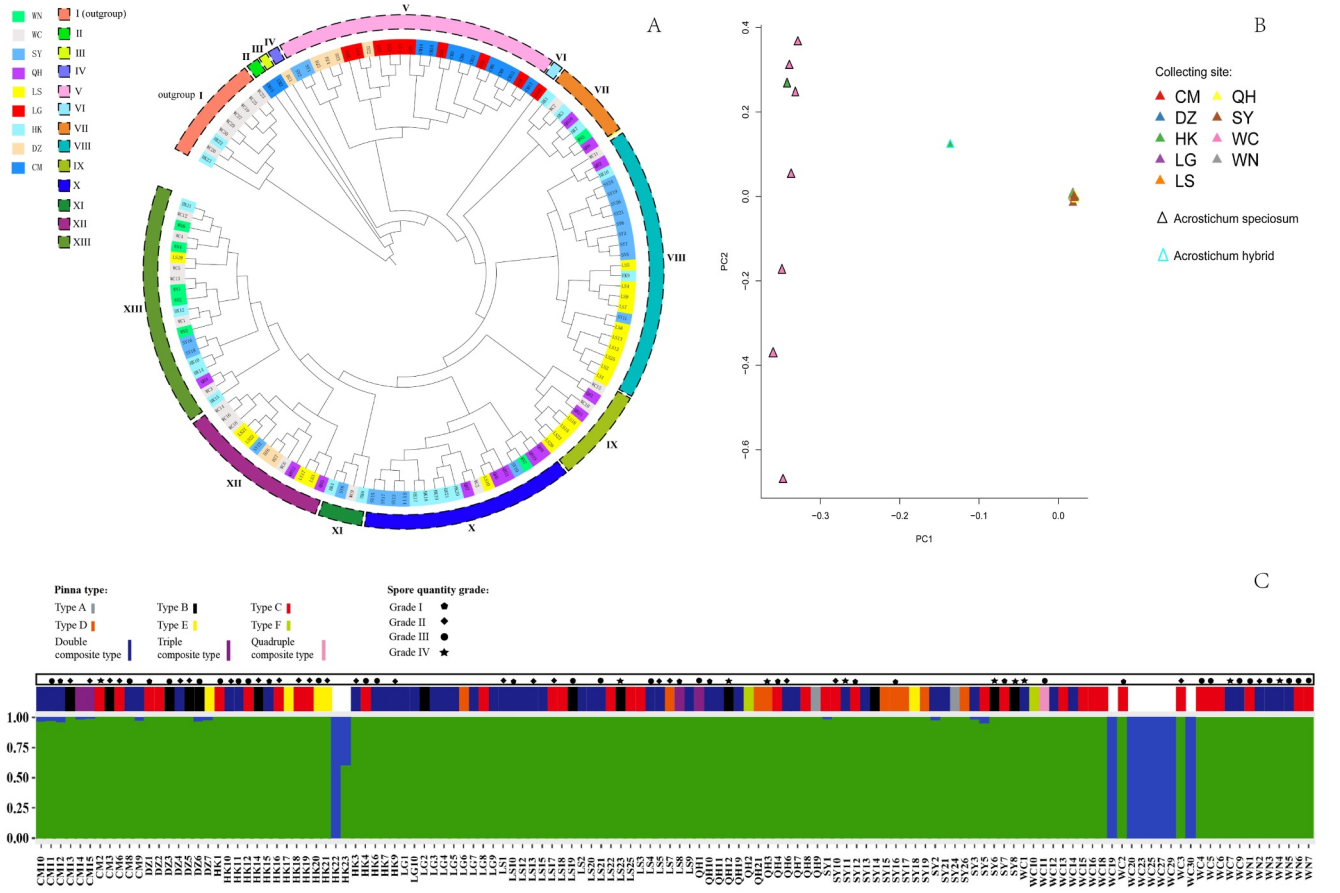


FIGURE 5 | Population genetic characteristics of *Acrostichum aureum* in Hainan Island. (A) Phylogenetic tree; (B) PCA analysis; (C) The population structure. HK22、WC19、WC20、WC23、WC25、WC27、WC29、WC30 are samples of *Acrostichum speciosum*;HK23 is a suspected hybrid sample.

confirming its hybrid origin between *A. aureum* and *A. speciosum*.

This integrated evidence strongly supports HK23 as a hybrid, while also indicating sporadic genetic introgression between *A. aureum* and *A. speciosum*.

3.3.3 | Population Genetic Diversity

The *A. aureum* samples were genetically structured into 12 distinct populations. Across these groups, the observed heterozygosity (*Ho*) ranged from 0.0771 to 0.1042 (mean = 0.0897), while the expected heterozygosity (*He*) varied between 0.0576 and 0.0865 (mean = 0.0660) (Table 2). Notably, all populations exhibited higher observed than expected heterozygosity, suggesting limited inbreeding. This was further supported by the inbreeding coefficient (*Fis*), which ranged from −0.0407 to 0.0017 (mean = −0.0243), indicating heterozygote excess and low inbreeding pressure. Despite their genetic subdivision, most pairwise *Fst* values among populations were below 0.05 (Supporting Information S2: Table S3), reflecting weak genetic differentiation. Additionally, nucleotide diversity (π) across all *A. aureum* populations ranged from 0.0647 to 0.0780 (mean = 0.0704), further supporting moderate genetic diversity with minimal population divergence. Pearson correlation analysis demonstrated that there are significant correlations among *Ho*, *He*, and π, but no significant correlation between π and *Fis* (Table 3).

4 | Discussion

4.1 | Genetic Diversity and Environmental Adaptation of *A. aureum* in Hainan Island

Based on SNPs (Figure 6, Supporting Information S2: Table S4), the π value of *A. aureum* in Hainan Island is much lower than that of two rare angiosperms *Rhododendron sinofalconeri* (vulnerable; [41]), *Michelia lacei* (endangered; [42]), and a critically endangered fern, *Adiantum nelumboides* [43]. The research by Zhang et al. [44] supports this result. They found minimal haplotype variation (1–2 haplotypes per population) in *A. aureum* using nuclear and chloroplast markers. Despite its lower diversity than some rare plants, *A. aureum*'s π value exceeds the typical range for threatened species (π = 0.00046–0.013) and even approaches that of nonthreatened plants (π = 0.0013–0.016) cited by Jing et al. [45]. The variation in diversity assessments may stem from sampling effects (e.g., number of populations/individuals sequenced, geographic coverage, and sequencing methods; Supporting Information S2: Table S4), and the evolutionary history of species [45].

The relatively low nucleotide diversity observed in Hainan Island's *A. aureum* populations stems from historical and contemporary environmental pressures. Pleistocene sea-level fluctuations triggered a genetic bottleneck in mangrove species [46], while modern anthropogenic impacts have exacerbated this condition. Coastal development has reduced Hainan's mangrove coverage by over 50% since the 1950s [7], with climate-change induced sea level rise causing particularly severe damage in Yalong Bay [13]. These disturbances have led to significant genetic erosion, with current populations likely descending from a limited ancestral group [44]. Parallel findings in *Alsophila* tree ferns [47] demonstrate how population

TABLE 2 | Genetic diversity of different populations of *Acrostichum aureum* in Hainan Island.

Population	*Ho*	*He*	π	*Fis*
CM	0.0771	0.0644	0.0678	0.0017
DZ	0.0780	0.0623	0.0676	−0.0110
HK-1	0.1042	0.0731	0.0767	−0.0407
HK-2	0.0843	0.0576	0.0647	−0.0329
LG	0.0795	0.0633	0.0671	−0.0080
LS-1	0.0969	0.0686	0.0723	−0.0338
LS-2	0.1025	0.0733	0.0780	−0.0380
QH	0.0906	0.0648	0.0677	−0.0304
SY	0.0953	0.0725	0.0746	−0.0087
WC-1	0.0916	0.0673	0.0714	−0.0237
WC-2	0.0881	0.0610	0.0671	−0.0359
WN	0.0879	0.0639	0.0694	−0.0296
Average	0.0897	0.0660	0.0704	−0.0243

TABLE 3 | Pearson correlation analysis of *Ho*, *He*, π, and *Fis*.

	Ho	*He*	π	*Fis*
Ho	1			
He	0.801**	1		
π	0.863**	0.968**	1	
Fis	−0.696*	−0.16	−0.319	1

Note: **At the 0.01 level (double tailed), the correlation is significant; *At the 0.05 level (double tailed), the correlation is significant.

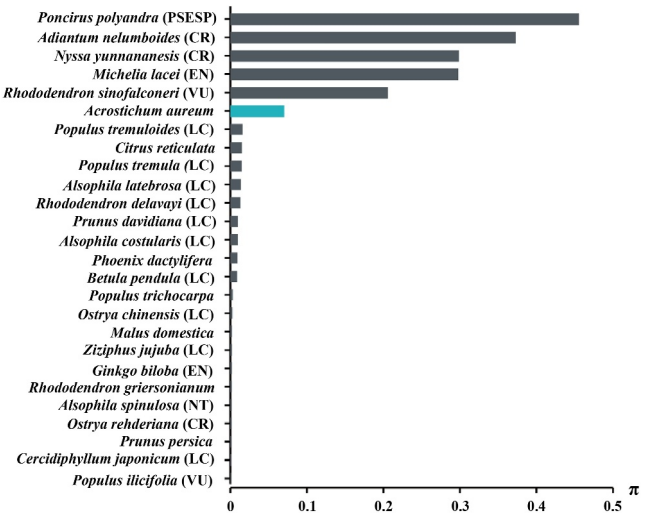


FIGURE 6 | Comparison of π values among representative plant species based on SNPs (CR-Critically Endangered; EN-Endangered; LC-Least Concern; NT-Near Threatened; VU-Vulnerable; PSESP-Plant Species with Extremely Small Populations).

contractions consistently reduce genetic diversity across fern species.

Despite its genetic constraints, *A. aureum* exhibits remarkable ecological plasticity. The species thrives across diverse habitats—from coastal estuaries to inland wastelands and shrimp ponds - demonstrating adaptive pinna morphology that correlates with local soil conditions [48]. This resilience stems from a dual reproductive strategy: rapid colonisation through clonal expansion via rhizomes, coupled with outcrossing that maintains heterozygosity ($Fis < 0$, $Ho > He$). While promoting widespread establishment, these mechanisms also limit genetic differentiation among populations.

Conservation priorities should focus on key genetic reservoirs. Based on the heterozygosity and π in this study (Table 2), Yanfeng Town (Haikou) and Yelin Town (Lingshui) with the population HK-1 and LS-2 respectively, are identified as diversity hotspots. Their heterozygosity and π are highest among the 12 populations. However, Hainan University (Haikou) with the population HK-2 represents a genetically vulnerable area, which has the lowest *He* and π values. Establishing protected zones in these locations would safeguard both the species' adaptive potential and remaining genetic variation. This approach recognizes the significance of the high genetic diversity of *A. aureum* for maintaining its evolutionary capacity amidst ongoing coastal changes.

4.2 | Relationships Between Spore Number, Reproductive Mode and Chromosome Ploidy of *A. aureum*

Our study reveals distinctive reproductive patterns in *A. aureum* that contrast markedly with related fern species in the same family Pteridaceae. While Nakato et al. [49] established that *Pteris* species typically exhibit apomictic reproduction when producing fewer than 32 spores per sporangium and sexual reproduction at 64 spores, we observed sexual reproduction in *A. aureum* specimens (e.g., LS8) with fewer than 32 spores. This fundamental difference likely reflects their contrasting habitats: *A. aureum* thrives in the dynamic intertidal zone where sexual reproduction maintains crucial genetic diversity for environmental adaptation, whereas terrestrial *Pteris* species favor the colonisation efficiency of apomixis in more stable habitats. These findings highlight how microenvironmental pressures can drive divergent reproductive strategies in ferns.

The conventional correlation between spore size and ploidy level, well-documented in *Pteris terminalis* [49, 50], proves unreliable for *A. aureum*. Our analysis of five diploid specimens (LS8, HK21, LS24, LS23, and WN4) demonstrated considerable variation in spore production (Grades I-IV) despite identical ploidy. This breakdown of the expected relationship suggests that the extreme conditions of intertidal environments, including fluctuating salinity and periodic inundation, may independently influence spore development, decoupling morphological traits from chromosomal characteristics. These results necessitate a paradigm shift in fern genetics research, emphasizing the need for direct ploidy assessment methods such as flow cytometry rather than relying on spore morphology in coastal species.

5 | Conclusion

Our multidisciplinary investigation of *A. aureum* across 12 populations in Hainan Island has yielded several key findings with important conservation implications. The morphological analysis revealed five distinct clades, demonstrating a clear correlation between pinna morphology and habitat types, though no such relationship was observed with spore production. Cytological examinations showed sexual reproduction occurring across specimens with varying spore counts, while establishing that chromosomal ploidy is independent of spore number, challenging conventional fern reproductive paradigms. Genetic analyses uncovered concerning patterns despite the species' wide distribution. The observed relatively low nucleotide diversity, coupled with the absence of significant population differentiation, suggests *A. aureum* maintains genetic variation primarily through gametophyte outcrossing rather than self-fertilization. This reproductive strategy, while promoting heterozygosity, may limit adaptive potential in the face of rapid environmental change. Three critical conservation areas were identified based on genetic diversity metrics, including high-diversity zones, such as Yanfeng Town (Haikou) and Yelin Town (Lingshui), and a genetic vulnerability zone, Hainan University (Haikou).

These findings carry broader ecological significance. As mangrove edge species, *A. aureum* populations serve as both ecological indicators and protective buffers for the entire mangrove ecosystem. Their apparent abundance belies underlying genetic vulnerability, which is a crucial lesson for mangrove conservation biology. The species' sensitivity to environmental changes, combined with its important ecosystem functions, necessitates focused protection efforts despite its current widespread distribution.

This study establishes that population size and distribution alone are poor proxies for genetic health in edge-adapted mangrove species. The results provide a framework for prioritizing conservation efforts in Hainan's mangrove ecosystems while offering methodological insights for studying other ecologically important but often overlooked marginal species in threatened habitats.

Author Contributions

Bingyan Guo: writing – original draft, investigation, formal analysis. **Rui Guo:** investigation, writing – original draft, formal analysis. **Hongjuan Zhu:** investigation, formal analysis, writing – original draft. **Changwang Ma:** investigation. **Dan He:** investigation. **Xialan Cheng:** investigation. **Yougen Wu:** writing – review and editing. **Ya Liu:** writing – review and editing. **Jing Yu:** writing – review and editing. **Dongmei Yang:** investigation, funding acquisition, writing – original draft, writing – review and editing, supervision.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The clean sequencing data of *A. aureum* have been stored in Genome Sequence Archive (GSA). The number of GSA is CRA011130. The genomic Illumina sequencing data, including 121 *A. aureum* samples (https://ngdc.cncb.ac.cn/gsa/).

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.

Supporting Information S1: bod270011-sup-0001-suppl-data.docx.

Supporting Information S2: bod270011-sup-0002-suppl-data.xlsx.