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MORPHOLOGICAL VARIATION IN SELECTED CITRUS SPECIES: A QUALITATIVE ANALYSIS OF SEED MORPHOLOGY AND FRUIT CHARACTERISTICS

O.A. BELLO AND O.M. ABIONA

Department of Biology, Osun State College of Education, Ila-Orangun, Nigeria

Corresponding Author*:nikebello73@gmail.com**; **Tel**:+234

ABSTRACT

Citrus species exhibit a wide range of morphological traits essential for taxonomy, genetic breeding, and germplasm conservation. This study assessed morphological variations in fruits and seeds of *Citrus grandis*, *Citrus limon*, and *Citrus medica*, focusing on colour differences and structural features. Fruit specimens were selected based on availability. Seed colour was analyzed using the Munsell Colour Chart. Ten seeds per species were evaluated and fruit structures were examined for the number of ridges and prominent lines. Colour variation in *C. grandis* ranged from White (8/2 2.5Y) to Yellowish Red (8/2 10YR); in *C. medica* from Yellowish Red (8/2 2.5YR) to Yellowish Red (8/2 10YR); and in *C. limon*, from Light Olive Brown (5/6 2.5Y) to White (8/2 2.5Y), showing a broader spectrum. Ridge and line counts varied significantly among species and within individuals. Qualitative colour analysis using the Munsell Chart proved to be a valuable complement to traditional morphological approaches. Results suggest that fruit and seed colouration are not only taxonomically significant but may also influence ecological interactions with dispersal agents.

Keywords: Citrus species, Colour, Dispersal, Morphology, Seeds, Variation

INTRODUCTION

The genus *Citrus* is one of the most economically and nutritionally important groups of fruit crops worldwide, belonging to the family Rutaceae. It encompasses a wide range of species such as *Citrus grandis* (pummelo), *Citrus medica* (citron), and *Citrus limon* (lemon), each bearing unique morphological, genetic, and phytochemical characteristics. Understanding the morphological variation among citrus germplasms is crucial for breeding programs, conservation strategies, and taxonomic classification.

The Rutaceae family, consisting of about 150 genera and over 1500 species, is distributed in both tropical and temperate regions, especially in Australia and South Africa. About 25 genera and over 80 species have been reported from India (Sharma, 2004), while in Bangladesh, 16 genera and 28 species are recorded (Ahmed *et al.*, 2009). Globally, the family is renowned for its juicy citrus fruits including oranges, lemons, and grapefruits. Rutaceous members are valued for their high vitamin C content and presence of bioactive alkaloids. Major genera and their approximate number of species include *Fagara* (250), *Zanthoxylum* (200), *Ruta* (60), *Glycosmis* (60), *Eriostemon* (32), *Atalantia* (18), *Citrus* (12), *Murraya* (12), and *Aegle* (3) (Sharma, 2004).

Ethnobotanical research on local floristic diversity and medicinal uses has gained momentum in recent years, particularly in South Asia. Studies by Alam *et al.* (2006), Arefin *et al.* (2011), Anisuzzaman *et al.* (2007), Khan and Huq (1975), Uddin *et al.* (2013), Rahman (2013), Rahman and Jamila (2015), Rahman and Sarker (2015), Rahman and Keya (2014), and Yusuf *et al.* (2006, 2009) provide significant insights into the regional application of Rutaceae members. Citrus fruits originated in Southeast Asia and spread globally during the middle Ages. They are now cultivated on every continent and represent a dominant segment of global fruit trade. The long shelf life of Citrus fruits' and high export potential as both fresh and processed juice products make them economically viable (Samson, 2016).

Botanically, citrus plants are small trees often bearing axillary single spines, with young angular branches and aromatic tissues. Leaves are alternate, simple or 1-foliolate, with articulated winged petioles. Inflorescences are racemose-corymbose or solitary. Flowers are generally bisexual with a cup-shaped calyx and thick linear petals. Stamens may be four to ten times the number of petals. Fruits are classified as berries (hesperidium), featuring a leathery exocarp, spongy mesocarp, and pulp-filled endocarp segments. Seeds are angular-obovoid, pale, and may contain single or multiple embryos (Swingle and Reece, 1967).

Morphological diversification in fruits is an evolutionary response to environmental pressures. Fruits provide protection to developing seeds and enhance dispersal efficiency through various mechanisms including animal attraction via colour and flavor. Human domestication has further diversified fruit characteristics, selecting for size, colour, texture, and taste (Spjut, 1994; Janick, 2005). An understanding of fruit and seed morphology is essential for sustainable agricultural practices and improved productivity. Morphological analysis thus plays a vital role in identifying species characteristics and advising farmers on propagation methods.

This study aimed to provide qualitative data on the seed and fruit morphological traits of selected citrus species to enhance breeding, classification, and conservation strategies.
In this study, selected *Citrus* species were evaluated for morphological variations using qualitative assessments of seed colour and quantitative analysis of fruit ridges and prominent lines. The fruits were randomly selected due to availability, and seeds from each fruit were carefully extracted and matched with the Munsell Colour Chart to determine their colour groupings.

The primary objectives of this study were to:
1. Determine the range of seed colour variation among selected *Citrus* species;
2. Determine the variability in fruit structure using count-based metrics such as the number of ridges and prominent lines;
3. Highlight the potential of combining qualitative and quantitative analyses in citrus morphological studies.

Fruits protect developing seeds from adverse environmental conditions and predation by animals during their premature stages, thereby enhancing seed survival rates.. However, fruits that contain nutrients and minerals can become favourite foods for animals and humans as part of a balanced diet. The energetic cost of producing fruits is paid for through subsequent seed dispersal, e.g., birds, mammals, and humans disperse seeds to different habitats where they can propagate. In some cases, the origin of morphological novelties or particular structures associated with fruits play an essential role in seed dispersal by wind, water and animals. Diverse colours and flavours of mature fruits and seeds attract animals that eat the fruit and aid seed dispersal. Thus, morphological variations of fruits have diversified considerably. Humans have also domesticated a wide range of plants as fruit crops with different sizes, shapes, colours, flavours, and textures.An understanding of fruits characterization and variation help in identifying and giving silvicultural advice to local farmers against the traditional methods of propagation for sustainable food and nutritional security. Fruit variation has been recognized as an immeasurable method of identifying species characteristics of many tropical fruits. This study aims to contribute to the improvement of Citrus fruit productivity and quality.

MATERIALS AND METHODS

Seeds were collected from Ila Orangun; Osun State from the fruits of *Citrus grandis*, *Citrus lemon* and *Citrus medica* (Plates 1, 2 and 3).The study was conducted to determine the seed morphological variations of *C. grandis*, *C. limon* and *C. medica* in *Citrus* family. Each fruit of *C. grandis*, *C. limon* and *C. medica* was cut into two equal halves, the seeds in the fruit were scooped out and the colour variation of the seeds were examined and matched with Munsell Colour Chart (2009).

Sample Collection

Fruits of three *Citrus* species—*Citrus grandis*, *Citrus medica*, and *Citrus limon* were randomly selected based on availability from local sources. Each species was represented by at least two fruits. The fruits were manually cut open to extract the seeds and observe the internal structural features.

Seed Colour Determination

Ten seeds from each species were carefully washed, air-dried, and matched against the Munsell Soil Colour Chart to determine precise colour classifications. This chart allows a standardized comparison of hue, value (lightness), and chroma (colour purity), which is especially useful in botanical colour identification (Munsell Colour, 2009).

Fruit Morphology

Each fruit was bisected to observe and count the number of ridges and prominent internal lines. Fruits of *C. grandis* were labeled CG1 and CG2; *C. medica* as CM; and *C. limon* as LM1 to LM3 for differentiation. The counts were tabulated and analyzed using the Chi-square statistical test to determine the significance of observed variation (Zar, 1999).

Plate 1 ***Citrus medica***

Plate 2 ***Citrus limon***

Plate3 ***Citrus grandis***

RESULTS

The seeds of *C. grandis*, *C. limon*, and *C. medica* showed different colours. A test of independence between *Citrus* species and colour type, at the ninety-five percent confidence interval, using Chi-square test gave a Chi-square value of 0.03, implying that the observed colour variation was species-dependent. This means that each species presented a different seed colour.

All the seeds of *C. grandis* were white. Seeds of *C. medica* were either white or pale yellow, while seeds of *C. limon* were white, yellow, pale yellow, or light olive brown (Figure 1). Seeds of *C. grandis* exhibited slight differences in the number of ridges and prominent lines. In Assertion 1, the number of ridges ranged from 3.00 to 16.00 and the number of prominent lines ranged from 1.00 to 3.00. In Assertion 2, ridges ranged from 5.00 to 11.00 and prominent lines from 3.00 to 7.00.

The colour of *C. grandis* seeds was not variable; it had the same colour code but varied in reference. The seed colour ranged from Yellow group (Y) to Yellowish Red group (YR), low range of (2.5Y) and high range of (10YR) 8/2 to 8/1. The seeds with the highest number of ridges also had the highest number of prominent lines and matched the Yellowish Red group at medium range of 8/1 10YR in Assertion 1 and 8/2 10YR in Assertion 2. Seeds with the least number of ridges and prominent lines matched Yellowish Red group at high range of 8/2 10YR in Assertion 1 and 8/2 2.5Y at low range in Assertion 2.

It seemed difficult to distinguish between the seed colours of Assertions 1 and 2, as they both had white colour and only differed in their reference numbers.

The colour of *C. medica* seeds differed from each other in colour code and reference number. Seeds at low range had the highest number of ridges and prominent lines, matching Yellowish Red group (2.5YR). Medium-range seeds matched Yellow group (5Y), and high-range seeds matched Yellowish Red group (10YR). Seed colour varied from 8/2 2.5YR White to 8/4 2.5YR Yellow at highest ridge number; 8/2 5Y White to 8/4 5Y Pale Yellow at medium range; and 8/1 10YR White to 8/2 10YR White at high range (Table 3).

Seeds of *C. limon* from LM1, LM2, LM3, and LM4 revealed slight differences in seed colour code and reference number (Table 4). LM1 seeds matched Yellow group (Y); LM2 matched Yellowish Red group (YR) at low range, similarly for LM3 and LM4. LM1 seeds varied from 5/6 2.5Y Light Olive Brown to 8/2 2.5Y White. LM2 varied from 7/4 2.5Y Pale Yellow to 8/3 2.5Y Pale Yellow and 8/2 2.5Y White. LM3 ranged from 7/2 2.5Y Light Gray to 7/3 2.5Y Pale Yellow and 8/2 2.5Y White. LM4 ranged from 7/3 2.5Y Pale Yellow to 8/2 2.5Y White. Seed colours in *C. limon* were distinguished by colour code, but all matched the Yellow group.

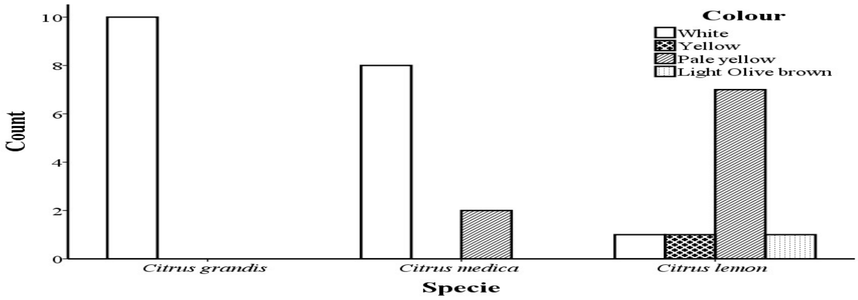

Figure 1: Colour of Seeds of the *Citrus* species

Table 1 Seed Morphological Traits of *Citrus grandis*

S/N	NUMBER OF RIDGES	PROMINENT LINES	SEED COLOUR
1	16	6	8/1 10YR WHITE
2	12	4	8/2 10YR WHITE
3	13	4	8/2 10YR WHITE
4	15	5	8/1 10YR WHITE
5	12	5	8/2 10YRWHITE
6	16	6	8/1 10YR WHITE
7	9	3	8/2 2.5Y WHITE
8	14	7	8/2 2.5Y WHITE
9	8	4	8/2 10YR WHITE
10	3	1	8/2 10YR WHITE

Table 2 Seed Morphological Traits of *Citrus grandis*

S/N	NUMBER OF RIDGES	PROMINENT LINES	SEED COLOUR
1	11	4	8/2 10YR WHITE
2	14	6	8/2 10YR WHITE
3	8	4	8/1 10YR WHITE
4	9	4	8/2 10YR WHITE
5	11	5	8/1 10YR WHITE
6	12	7	8/2 10YR WHITE
7	9	5	8/2 2.5Y WHITE
8	5	3	8/2 2.5Y WHITE
9	8	4	8/2 10YR WHITE
10	7	5	8/2 2.5Y WHITE

Seed coat colour of *Citrus grandis* varied in Munsell colour coded from Yellowish Red (YR) to Yellow (Y), although in both Assertions 1 and 2; these codes visually matched a white seed colour. Specifically, seed coat colours ranged from 8/1 10YR to 8/2 10YR at the higher spectrum, and from 8/2 2.5Y at the lower spectrum in both assertions (Tables 1 and 2). Seed colour of C*, grandis* were 8/2 10YR WHITE, 8/1 10YR WHITE and 8/2 2.5Y WHITE (Table 1)

Number of Ridges and Prominent Lines
The number of seed ridges in *C. grandis* ranged from 3 to 16 in Assertion 1, and from 5 to 14 in Assertion 2 (Table 3). Number of prominent lines observed ranged from 1 to 7 in Assertion 1 and from 3 to 7 in Assertion 2. In both cases, the maximum number of prominent lines was 7.

Table 3: Seed Morphological Traits of *C. medica*

S/N	NUMBER OF RIDGES	PROMINENT LINES	SEED COLOUR
1	15	7	8/1 10YR WHITE
2	11	5	8/2 10YR WHITE
3	12	5	8/1 10YR WHITE
4	12	7	8/2 10YR WHITE
5	16	6	8/2 2.5YR WHITE
6	13	5	8/4 2.5YR YELLOW
7	15	5	8/2 2.5YR WHITE
8	12	4	8/2 5Y WHITE
9	13	5	8/3 5Y PALE YELLOW
10	10	5	8/4 5Y PALE YELLOW

Seed Colour Variation in *Citrus limon*

Seeds of *Citrus limon* exhibited noticeable colour variation when assessed using the Munsell Colour Chart (2009), across four sampled fruits labeled LM 1 to LM 4 (Table 4). In LM 1, seed colours ranged from White, Yellow, Pale Yellow, Light Yellow to Light Brown (Table 4). LM 2 showed variation from White to Pale Yellow (Table 5). LM 3 included Light Gray, White, and Pale Yellow, while LM 4 ranged from Pale Yellow to White (Table 6). While all *C. limon* types consistently presented White and Pale Yellow seeds, LM 1 uniquely displayed Light Olive Brown, Light Brown (Table 4), and Yellow seeds (Table 5), and LM 3 showed Light Gray (Table 6). The White seed colour was consistently referenced as 8/2 2.5Y across all four fruit samples. Pale Yellow shades varied slightly in reference codes, including 7/3, 7/4, and 8/3 2.5Y (Table 7).

Table 4 Seed Morphological Traits of *C.limon*. (LM 1)

S/N	SEED COLOUR	REFERENCE NUMBER
1	WHITE	8/2 2.5Y
2	YELLOW	7/6 2.5Y
3	PALE YELLOW	7/4 2.5Y
4	PALE YELLOW	7/4 2.5Y
5	LIGHT OLIVE BROWN	5/6 2.5Y
6	PALE YELLOW	7/4 2.5Y
7	LIGHT BROWN	8/6 2.5Y
8	WHITE	8/2 2.5Y
9	PALE YELLOW	7/4 2.5Y
10	PALE YELLOW	7/4 2.5Y

Table 5 Seed Morphological Traits of *C.limon*. (LM2)

S/N	SEED COLOUR	REFERENCE NUMBER
1	WHITE	8/2 2.5Y
2	WHITE	8/2 2.5Y
3	PALE YELLOW	8/3 2.5Y
4	WHITE	8/2 2.5Y
5	WHITE	8/2 2.5Y
6	PALE YELLOW	8/3 2.5Y
7	PALE YELLOW	7/4 2.5Y
8	PALE YELLOW	7/3 2.5Y
9	WHITE	8/2 2.5Y
10	WHITE	8/2 2.5Y

Table 6 Seed Morphological Traits of *C.limon*. (LM 3)

S/N	SEED COLOUR	REFERENCE NUMBER
1	LIGHT GRAY	7/2 2.5Y
2	WHITE	8/2 2.5Y
3	WHITE	8/2 2.5Y
4	PALE YELLOW	7/3 2.5Y
5	PALE YELLOW	7/3 2.5Y
6	WHITE	8/2 2.5Y
7	WHITE	8/2 2.5Y
8	PALE YELLOW	7/4 2.5Y
9	PALE YELLOW	8/2 2.5Y
10	WHITE	8/2 2.5Y

Table 7 Seed Morphological Traits of *C.limon*. (LM 4)

S/N	SEED COLOUR	REFERENCE NUMBER
1	PALE YELLOW	7/3 2.5Y
2	WHITE	8/2 2.5Y
3	WHITE	8/2 2.5Y
4	PALE YELLOW	7/4 2.5Y
5	PALE YELLOW	7/4 2.5Y
6	PALE YELLOW	7/4 2.5Y
7	WHITE	8/2 2.5Y
8	WHITE	8/2 2.5Y
9	WHITE	8/2 2.5Y
10	PALE YELLOW	7/3 2.5Y

Variation in Number of Ridges and Prominent Lines in Citrus Species

Number of ridges observed on the seeds from the second assertion (half) of *Citrus grandis* did not differ significantly from those found in *C. medica* (Table 8). Similarly, the number of prominent lines on the same set of seeds from *C. grandis* was not statistically different from that of *C. medica*, at a 95% confidence level.

Plate 4: Number of Ridges on *Citrus* species

Table 8: Chi-Square Test between *C. grandis (*Assertion 2) seeds and *C. medica* seeds

SN	Variable	Chi Square Value	Degree of Freedom	p-value	Remark
1	Number of Ridges	14.333	10	0.158	Not statistically different
2	Number of Prominent Lines	4.133	4	0.388	Not statistically different

Statistical Analysis of Ridges and Prominent Lines between Citrus grandis and C. medica

The number of ridges observed on seeds from the first assertion of *C. grandis* did not differ significantly from those of *C. medica*, as the computed p-value was 0.616—substantially higher than the standard significance threshold of 0.05 (Table 9). There was no significant difference in the number of prominent lines between the seeds of *C. grandis* and *C. medica*. These findings indicate that neither the number of ridges nor the number of prominent lines is species-dependent within the context of this study.

Table 9: Chi-Square Test between *C. grandis* (Assertion 1) and *C. medica* seeds

SN	Variable	Chi Square Value	Degree of Freedom	p-value	Remark
1	Number of Ridges	7.200	9	0.616	Not statistically different
2	Number of Prominent Lines	5.667	5	0.340	Not statistically different

Comparison Between First and Second Assertions of Citrus grandis

There was no statistically significant difference in the number of ridges between the two assertions at the 90% confidence interval (Table 10). Number of prominent lines observed on the seeds from both assertions did not differ significantly.

Table 10: Chi-Square Test between *C. grandis* Assertion 1 and 2 seeds

SN	Variable	Chi Square Value	Degree of Freedom	p-value	Remark
1	Number of Ridges	10.000	10	0.440	Not statistically different
2	Number of Prominent Lines	1.676	5	0.892	Not statistically different

DISCUSSION

The morphological variability in *Citrus* species, particularly with respect to seed characteristics such as colour, ridges, and prominent lines, has significant implications for the taxonomy and ecological roles of these plants. The findings of this study provide valuable insights into the seed morphology of *Citrus grandis*, *Citrus medica*, and *Citrus limon*, particularly in terms of colour and structural features. The results revealed considerable variation in seed colour, but no significant differences in the number of ridges and prominent lines between species or assertions of the same species.

Seed Colour Variation and Ecological Implications

The seed colour of the three *Citrus* species studied exhibited notable diversity. *Citrus limon* demonstrated the broadest range of colour variations, including white, pale yellow, light olive brown, and light gray, while *C. grandis* and *C. medica* displayed a more limited range of colourations, primarily varying between yellowish red and yellow groups. These findings align with earlier studies of Krueger & Navarro, 2007 that indicated that colour diversity in seeds could be influenced by both genetic and environmental factors.

The observed seed colour patterns in this study also reflect the ecological importance of seed colour in *Citrus* species. As bright and contrasting colours often aid in seed dispersal, it is likely that these variations play a role in attracting seed dispersers, such as birds and humans, which are primary vectors for *Citrus* seeds (Schaefer *et al.*, 2004). Although citrus seeds are enclosed within the fruit and not immediately visible to primary dispersers, the diversity in seed colour may reflect underlying genetic, physiological, or ecological factors important for species differentiation and post-dispersal success. Seed colour has been associated with indicators of maturity, dormancy, and viability (Bewley *et al.*, 2013). Once fruits are consumed by animals or undergo natural decay, seeds become exposed, and their coloration may aid in detection by secondary dispersers or influence microhabitat selection for germination (Willson & Traveset, 2000). Therefore, while seed colour in citrus may not directly enhance primary dispersal through visibility, it likely contributes to broader ecological and evolutionary functions related to survival, dispersal efficiency, and genetic diversity. The results also suggest that climatic factors, soil type, and maturity levels may influence seed colour variation. This hypothesis is supported by previous research (Ladanyia, 2010), which indicated that such traits are subject to environmental modifications.

Ridges and Prominent Lines: Structural Traits

In contrast to seed colour, the number of ridges and prominent lines on *Citrus* seeds did not exhibit significant variation across species or between assertions of the same species. There were no significant differences in the number of ridges or prominent lines between *C. grandis* and *C. medica*, or between the two assertions of *C. grandis*. These findings suggest that these morphological traits are not reliable indicators for distinguishing between species within this genus. Furthermore, lack of significant differences implies that ridge and line features may be more influenced by factors unrelated to species identity, such as environmental conditions or genetic variability within species.

Previous studies on the internal fruit anatomy of *Citrus* species (Scora, 1975; Nicolosi *et al.*, 2000) have also pointed out that structural traits such as ridges and prominent lines can serve as markers for classification. However, the results of this study show that these traits may not always provide sufficient resolutions for species identification in certain contexts, particularly when viewed in isolation.

Implications for Germplasm Evaluation and Conservation

The findings from this study highlight the importance of integrating qualitative tools, such as the Munsell Colour Chart, with traditional methods for evaluating *Citrus*germplasm. The Munsell Colour Chart, which provides a standardized approach for assessing seed colour, proved to be an effective tool for complementing traditional morphological analyses. This integration of methods can enhance the precision of plant characterization, particularly for germplasm evaluation, which is crucial for breeding, conservation, and sustainable cultivation practices.

Lack of significant morphological variation between species in traits like ridges and prominent lines emphasizes the need for comprehensive approaches in the study of *Citrus* biodiversity. Future research should focus on a broader range of species, include genetic analyses, and consider environmental factors that may contribute to phenotypic variation.

The results of this study underscore the complexity of seed morphology in *Citrus* species. While seed colour demonstrated significant variation, structural traits such as ridges and prominent lines showed little to no variation between species or assertions. These findings contribute to understanding of *Citrus* taxonomy and offer insights into the ecological roles of seed characteristics. The study also highlights the utility of combining traditional morphological techniques with modern tools, such as the Munsell Colour Chart, to enhance the accuracy of plant characterization.

CONCLUSION

This study provides valuable insights into the morphological variation of selected *Citrus* species, with emphasis on seed colour and structural features. The application of the Munsell Colour Chart revealed that seed colour ranges from white and pale yellow to light brown and yellowish-red, with notable intra- and interspecific variability. This diversity in seed coloration, although the seeds are enclosed within the fruits, may play a role in ecological adaptation and post-dispersal identification by animals once fruits are split or decayed. On the other hand, structural traits such as the number of ridges and prominent lines on the fruit surface showed less variability, suggesting greater taxonomic stability in those characteristics.

The observed variations could be influenced by environmental factors such as climate, soil type, and fruit maturity stages, consistent with previous findings (Ladanyia, 2010). These morphological traits, though subtle, contribute to taxonomic differentiation, genetic conservation, and breeding program design. This study also reinforces the utility of integrating traditional observation with standardized colourimetric tools like the Munsell Colour Chart for accurate plant identification and classification.

Ultimately, understanding fruit and seed morphology in *Citrus* species not only supports botanical and genetic research but also provides practical benefits in agricultural productivity, varietal selection, and the promotion of sustainable citrus cultivation.

Further research into the genetic and environmental influences on seed morphology will provide a more comprehensive understanding of *Citrus* diversity and its implications for breeding and conservation efforts.

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