TAPROBANICA, ISSN 1800–427X. November, 2024. Vol. 13, No. 02: pp. 138–140, pls. 30–31.

http://www.taprobanica.org
https://doi.org/10.47605/tapro.v13i2.341

Genetic diversity and population structure of *Crudia zeylanica* (Fabaceae), Sri Lanka

The genus *Crudia* (Schreb.) belongs to the family Fabaceae and is named after J.W. Crudy (1753–ca.1810), a plant collector from the Bahamas (Lanjouw & Stafleu 1954). The genus is pantropical and consists of approximately 55 species, mostly from Asia with others from Africa and tropical America. The species was first discovered and originally described as *Detarium zeylanicum* (Thwaites, 1864) and assigned to the genus *Crudia* in 1865 (Bentham 1865). The holotype was collected from Galpatha, Kaluthara District, and deposited at the National Herbarium of Peradeniya under C.P number 3714. Since then, there have been no further records of this species from the field and it has been categorised as extinct species in the IUCN Red List of 2006 and the National Red List of 2012. *Crudia zeylanica* was rediscovered in 2019 when a mature tree was identified based on its morphology in the Gampaha District of Sri Lanka. Subsequent explorations in the same district identified seven populations consisting of mature trees, saplings, and seedlings. With these findings, the National Red List of 2020 categorized *C. zeylanica* as a Critically Endangered (CR) species (Wijesundara *et al.* 2020). Further, it is a protected species under the provisions of the Fauna and Flora Protection Ordinance of Sri Lanka.

Crudia zeylanica is a large tree that grows close to water sources such as rivers, marshes, and canals of wet lowlands. *Crudia zeylanica* leaves imparipinnate with 2–6 leaflets; leaflets asymmetrical alternate, ovate to sub-ovate, base rounded, apex obtuse with a short-rounded terminal protrusion, about 5–15 cm long, 2–5 cm wide, glabrous on both surfaces, flowers small in spicate terminal racemes and reported to be flowering from December to January (Dassanayake 1991).

Identifying genetic diversity is the essential first step in any sustainable conservation efforts. Proper propagation and multiplication strategies also depend on the true genetic diversity of a species. Interestingly, despite the remarkable biological diversity in Sri Lanka, *C. zeylanica* is confined to one Province and present in small, isolated populations.

Immature leaves were collected from each population of *C. zeylanica* (Fig. 1) in Sri Lanka (Sup. Table 1). Herbarium specimens were prepared following the standard protocol and deposited at National Herbarium, Royal Botanic Gardens, Sri Lanka. The GPS coordination of the collection sites was recorded using GARMIN GPSmap 78s and mapped using ARC GIS version 10.8.1 ESRI, 2020 (Fig. 2).

Total genomic DNA from leaf samples were extracted using QIAGEN DNeasy® Plant Mini (Cat. No.: 69104) following the manufactures guidelines. The polymerase chain reaction (PCR) was performed using standard universal plant DNA barcoding primers (Sup. Table 2). PCR was carried out using the previously optimized protocol for universal barcoding work for plants with high phenolics (Chandrasekara *et al.* 2021). Briefly, 25 µL reaction volume consisted of lx PCR buffer, 1.5 mM MgCl2, 200 µM dNTP (Promega, Cat No: U1515), 0.2 µM of each primer (Integrated DNA Technologies, Singapore), 100 ng of DNA, 0.8 µM spermidine and 1 Unit Go Taq Flexi DNA polymerase (Promega, Cat No: M8295). The PCR machine programmed for 94 °C of initial denaturation for 2 minutes, followed by 35 cycles of 94 °C for 1-minute, annealing temperature ranging from 55 °C for 30 seconds (depending on primer) and 72 °C for 30 seconds, and final extension at 72 °C for 3 minutes. Amplified products were separated

by electrophoresis (5 V cm-1) on 1.5 % agarose gels and stained with Ethidium Bromide. Sanger sequencing was performed at Macrogen Inc. (Seoul, South Korea—www.dna.macrogen.com) using the same primers used for PCR.

After receiving the sequence data from Macrogen, sequencing chromatograms were visually inspected using Geneious Prime software for sequencing errors. The 5' and 3' noisy sequences of about 30 bp were removed and 535, 390, and 744 bp were obtained for rbcL, trnH_psbA and matK regions respectively. The multiple sequence alignments were done separately for each barcode region to check the sequence variation between samples.

Further, rbcL, matK, and trnH-psbA sequences of *Crudia* species were downloaded from the NCBI (Sup. Table 3) and multiple sequence alignment was carried out to check the most varying region among these three regions.

No sequence variation for rbcL gene among 12 *C. zeylanica* samples. There are four single nucleotide variations among NCBI samples (Sup. Table 4). No sequence variation for trnH-psbA intergenic region among 12 *C. zeylanica* samples. There are 12 single nucleotide variations among NCBI samples. There are 92 variations between *C. zeylanica* and NCBI samples. In addition to that, NCBI BLAST search was carried out against each sequence to compare nucleotide sequences to sequence databases and calculate the statistical significance of matches.

PCR carried out for rbcL and trnH-psbA regions was successful. Unfortunately, PCR for matk region worked only for two samples (no 3 and 16). RbcL and trnH-psbA sequences of all the samples and matK sequences of no 3 and 16 are given below (Sup. Table 5). Multiple sequence alignment analysis of rbcL and trnH-psbA revealed that all the sequences are identical except sample no 16 which is morphologically different too. There are 4 variable sites in rbcL and huge variation in trnH-psbA including insertions and deletions for no 16. matK sequence alignment between the two samples (No 3 and 16) also found they are different from each other.

The comparison carried out with available sequences of different *Crudia* species found less variation in rbcL region (4 variable sites in 528 bp) compared to trnH-psbA and matK (16/425 and 27/1518 variable sites respectively) (Sup. Table 6). This means that per-base pair variation is highest in trnH-psbA compared to rbcL and matK. Further, there are one and 13 variable sites that were found within three samples of *C. bracteata* and three *Crudia* aromatic species respectively while there were no variable sites among two samples of *C. oblonga* species for matK region. This finding further supports that the variation found in trnH-psbA is sufficient enough to distinguish species.

NCBI BLAST search carried out against each sequence found different hits including *Crudia* species for rbcL. However, *Crudia* hits were not found for trnH-psbA and matK sequences within the first 100 hits although trnH-psbA and matK sequences of *Crudia* are available in NCBI. This is because of huge variation between *C. zeylanica* and other *Crudia* species.

As *C. zeylanica* is an island population restricted to Sri Lanka, its genetic diversity is expected to be lower compared to mainland populations of related species. The geographic isolation and limited population size make it more susceptible to genetic drift, resulting in lower genetic variation. This aligns with the observation that island populations generally have less genetic diversity than their mainland counterparts due to their smaller population sizes and isolation. Being an endangered species, *C. zeylanica* likely experiences the effects of a small population size, which is to reduce its evolutionary potential. The limited number of individuals increases the likelihood of genetic drift and inbreeding, leading to higher homozygosity and a reduced ability to adapt to environmental changes. This is a well-established phenomenon in small populations of wild plant species, where genetic diversity is crucial for long-term survival. Unlike widespread species, which tend to maintain higher levels of genetic variation due to larger and more interconnected populations, *C. zeylanica* is geographically restricted and has a fragmented distribution. This makes it prone to genetic erosion, a process in which genetic diversity declines over time due to the small population size and limited gene flow between populations.

The endangered status of *C. zeylanica* suggests that it faces significant genetic challenges. As documented in studies like that of Szczecińska *et al*. (2016), dwindling populations often exhibit lower genetic diversity due to faster genetic drift, exacerbated by limited gene flow. This is especially relevant for *C. zeylanica*, which exists in a highly specific and isolated habitat. Limited genetic exchange between fragmented populations increases the risk of

Plate 30

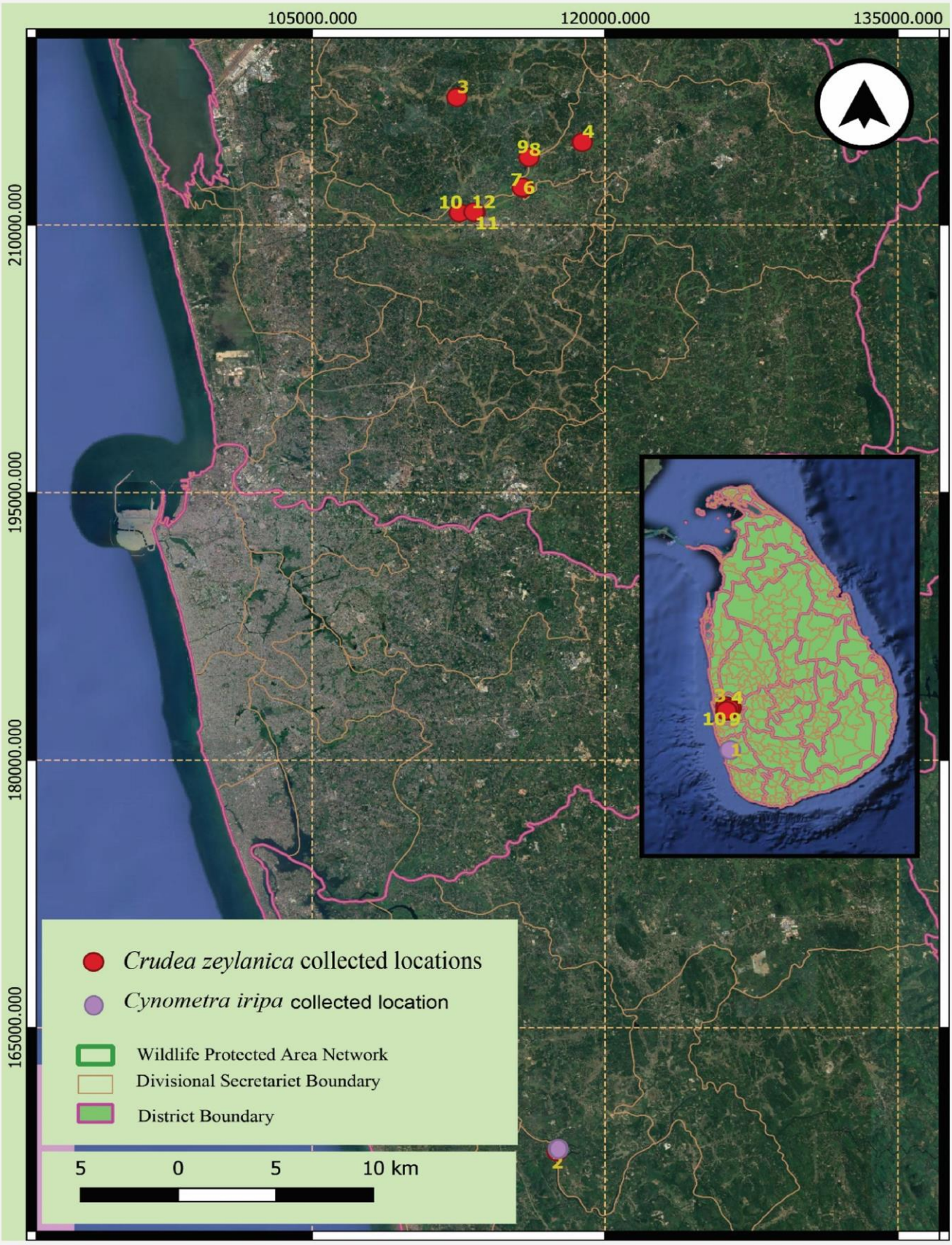

Figure 1. Locations of *Crudia zeylanica* samples collected in Gampaha District; *Cynometra iripa* sampled from Kaluthara District, Sri Lanka

Plate 31

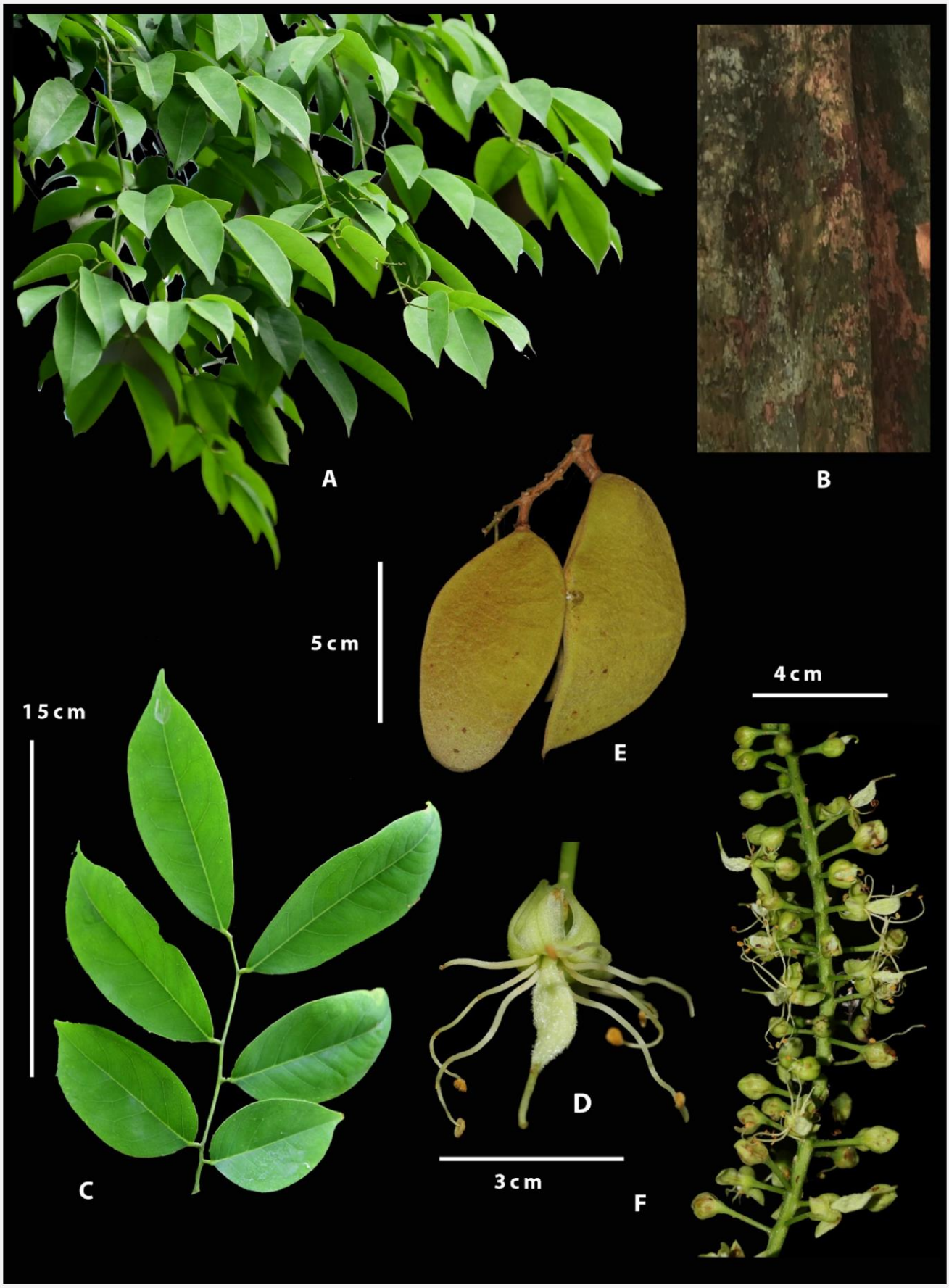

Figure 2. *Crudia zeylanica* (Thwaites) Benth. **(A)** branch, **(B)** texture of the trunk, **(C)** leaflet, **(D)** flower, **(E)** fruit, **(F)** inflorescence; © Photo: N. Jayawardena

inbreeding and lowers the species' adaptive potential.

A rare species, *C. zeylanica* may be experiencing inbreeding depression, which is characterized by reduced fitness and adaptive potential due to increased homozygosity. This can further threaten its survival, especially in the face of environmental stressors and habitat degradation. The genetic erosion hypothesis posits that species under stress—such as habitat fragmentation or population decline—experience a reduction in genetic diversity. This is particularly applicable to *C. zeylanica*, where the small, isolated populations are vulnerable to genetic drift and environmental changes, leading to a further loss of genetic variation over time. This decline in genetic diversity is closely tied to the species' limited capacity for adaptation and recovery, especially in unstable ecosystems. Given the above factors, the aim of estimating the genetic diversity within Sri Lankan populations of *C. zeylanica* is crucial for understanding the species' evolutionary potential and fitness. By examining the genetic structure of different populations, conservation efforts can better assess the level of genetic erosion and take measures to enhance gene flow, reduce inbreeding, and ultimately increase the species' chances of survival.

In summary, the genetic challenges faced by *C. zeylanica*, as an endangered, island-endemic species with small and isolated populations align with broader patterns of reduced genetic diversity seen in endangered and island species. Studying its genetic diversity can provide valuable insights into its conservation needs and potential for long-term survival. Genetic diversity and population structure are crucial elements in the conservation of endangered species, as they influence a population's capacity to adapt to environmental changes and ensure long-term survival. For species like *C. zeylanica*, which are already at risk due to small population size and limited distribution, understanding and preserving genetic diversity can provide the foundation for successful conservation and recovery strategies.

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Submitted: 11 Jul 2023, *Accepted*: 10 Jul 2024
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