



## Evaluating the Efficacy of Three DNA Barcodes in Identifying the Medicinal Species *Ceropegia lodarensis* (Lavranos) Bruyns (Apocynaceae)

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**C**EROPEGIA *lodarensis* (= *Huernia lodarensis*) is a succulent plant with medicinal uses in Arabian folk medicine. It belongs to *Ceropegia* sect. *Huernia*, the most diverse and widespread section of the highly succulent plants informally known as stapeliads. Morphological identification of this species is difficult and often impossible without flowers. This study tested the performance of three DNA barcodes to identify *C. lodarensis* at the molecular level. These DNA barcodes are: the nuclear ribosomal internal transcribed spacer (ITS) region, plastid *psbA-trnH* intergenic region, and noncoding plastid *trnL-trnF*. The identification was done using BLASTn searches; and tree-based methods, namely maximum likelihood (ML), maximum parsimony (MP) and neighbour joining (NJ). Results showed that the candidate regions have a low species-level resolving ability. However, ITS has the highest identification success rate at the sect. *Huernia* level, suggesting its potential for plant identification systems of *Huernia* species at the section level. Additional work is needed to examine the utility of further DNA loci.

**Keywords:** *Huernia*, ITS, Medicinal plants, Molecular identification, *psbA-trnH*, *trnL-trnF*.

### Introduction

*Ceropegia* L. s.l. (Ceropegieae, Asclepiadoideae, Apocynaceae) is a genus of approximately 725 species native to the Old World, ranging from southern Africa to northern Australia (Bruyns et al., 2020). Based on its expanded concept, *Ceropegia* includes 141 species of *Brachystelma* and 357 species of stapeliads, a group of 31 genera of leafless succulent stem plants (Bruyns et al., 2017). It has 63 sections after all stapeliads genera are reduced into sections and *Brachystelma* species transfer into several sections (Bruyns et al., 2017). *Ceropegia* sect. *Huernia* (formerly genus *Huernia* R.Br.) is the most diverse section within the stapeliads and consists of over 54 species widely distributed in the southern Arabian Peninsula and sub-Saharan Africa (Bruyns, 2008; Bruyns et al., 2017). Members of sect. *Huernia* are highly valued as medicinal plants (Teklehaymanot & Giday, 2007; Amoo et al., 2012; Schmelzer & Gurib-Fakim, 2013), ornamental succulents (Al-Turki, 2002), and famine food plants (Guinard & Lemessa, 2001).

*Ceropegia* sect. *Huernia* is represented in Saudi Arabia by four species: *Ceropegia khalidbinsultanii* (Plowes & McCoy) Alharbi & Al-Qthanin, *Ceropegia laevis* (J.R.I. Wood) Bruyns, *Ceropegia lodarensis* (Lavranos) Bruyns and *Ceropegia macrocarpa* (Sprenger) Bruyns, the most common of which is *C. lodarensis* (Alharbi & Al-Qthanin, 2021). *C. lodarensis* (= *Huernia lodarensis* Lavranos) is a complex group, and the presence of flower-colour polymorphism has led to the classification of several members as distinct species.

A recent taxonomic treatment of sect. *Huernia* in Saudi Arabia has resulted in the reduction of four names under *C. lodarensis*: *Huernia saudiarabica* D.V. Field, *Huernia collenetiae* Plowes, *Huernia foetida* Plowes and *Huernia rubrosticta* Plowes (Alharbi & Al-Qthanin, 2021). Several species of sect. *Huernia* in the Arabian Peninsula have shown promising ethnopharmacological potential (Ali et al., 1984; Hamam et al., 2018; Al-Fatimi, 2019). For example, pregnane glycosides and flavonoids from *C. lodarensis* (=

*H. saudi-arabica*) have been reported to have anti-schistosomal, anti-diabetic, and anti-obesity properties (El Sayed et al., 2018, 2020). The species ( $\equiv$  *Huernia lodarensis*) is also used in folk medicine to treat chronic wounds and injuries (Al-Fatimi, 2019).

Morphological identification of *C. lodarensis* is challenging in the absence of flowers because many species of sect. *Huernia* lack distinct vegetative traits. The sect. *Huernia* are dwarf, leafless angled succulent stems with creeping or mat-forming habits and inflorescence emergence near the base of the stem (Albers & Meve, 2002; Bruyns, 2005) (Fig. 1). Traditional plant identification using taxonomic keys is difficult and time-consuming. As the taxonomic keys are built mainly on floral characteristics (Bruyns et al., 2017; Alharbi & Al-Qthanin, 2021), they cannot be used to identify specimens lacking flowers. Moreover, sterile specimens are difficult to distinguish, particularly for nonspecialists, from the most closely related stapeliads sections, such as *Dovalia* and *Orbea* (Bruyns, 2005). Flowering times in stapeliads are mostly linked to rainfall, which often follows erratic patterns in stapeliads habitats (Bruyns, 2005). Thus, an accurate and highly effective identification tool for sterile and juvenile specimens of sect. *Huernia* species must be developed to avoid misidentification of these medicinal species.

DNA barcoding provides a powerful, rapid and practical tool for species identification in plant materials that may lack obvious morphological or anatomical diagnostic features (Hebert et al., 2003; Kress et al., 2005). The technique is based on the nucleotide diversity of sequences of a short and standardised DNA segment (Kress et al., 2005).

Although various DNA loci have been proposed and investigated either alone or in combination, a universal plant barcode has not been agreed upon (Vijayan & Tsou, 2010; Hollingsworth et al., 2011). However, scientists have agreed on the four regions used in most applications: two plastid coding genes (*rbcL* and *matK*), the plastid *psbA-trnH* intergenic region, and the nuclear ribosomal internal transcribed spacer (ITS) region (Kress et al., 2005; Kress & Erickson, 2007; CBOL, 2009).

A considerable amount of data for the stapeliads group generated in previous phylogenetic studies are contained in GenBank for several DNA regions (Bruyns et al., 2014; Meve et al., 2017; Fouad et al., 2019; 2022). Among these regions, three (ITS, *psbA-trnH*, and noncoding plastid *trnL-trnF*) are fast-mutating within Apocynaceae (Meve & Liede, 2002; Livshultz et al., 2007; Gathier et al., 2013; Selvaraj et al., 2015) and have been proposed as plant DNA barcodes (Chase et al., 2005; Kress et al., 2005; Taberlet et al., 2007). These data in GenBank can be utilised as reference material to test the molecular identification ability of sect. *Huernia* species. This study tests the effectiveness of the DNA barcoding approach in the molecular differentiation of *C. lodarensis* using nuclear ITS and plastids *psbA-trnH* and *trnL-trnF*.

## Materials and Methods

### Taxon sampling

Plant specimens of *C. lodarensis* were collected from the Ash Shafa area (21°5.5702'N, 40°21.785'E) in the Al-Taif region, Saudi Arabia, and the voucher specimens were deposited at the Umm Al-Qura University herbarium, Makkah, Saudi Arabia, with accession numbers S18a and S6B.

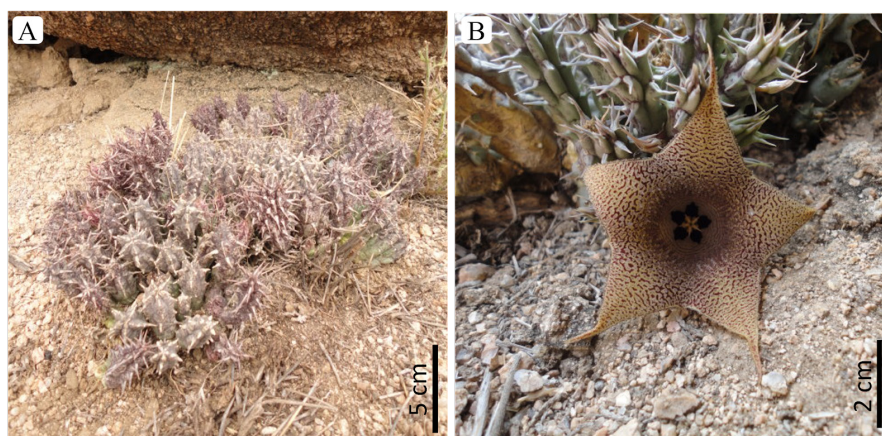


Fig. 1. *Ceropegia lodarensis* (A) mat-forming habit, (B) flowers emerging near the base of the stem [Photo by the author from Al-Taif region, Saudi Arabia]

In addition, a total of 245 accessions of ITS (81), *psbA-trnH* (83) and *trnL-trnF* (81) were obtained from GenBank. These accessions were previously generated in an evolutionary study of the stapeliads (Bruyns et al., 2014). The accessions represented 83 species from eight stapeliads sections, which correspond to the stapeliads genera recorded by Colletete (1999) in Saudi Arabia. The name of each section, along with the number of species obtained, the total number of species, and the percentage of species obtained are as follows: sect. *Apteranthes*: 2/6 (33%), sect. *Caudanthera*: 2/3 (66%) (data available for *psbA-trnH* only), sect. *Desmidorchis*: 11/23 (47%), sect. *Duvalia*: 7/16 (43%), sect. *Edithcolea*: 1/4 (25%), sect. *Huernia*: 24/54 (44%), sect. *Orbea*: 31/53 (58%) and sect. *Rhytidocaulon*: 3/15 (20%). Sequences retrieved from the National Center for Biotechnology Information (NCBI) database followed the traditional names of stapeliads genera and were used here without modification.

#### DNA extraction, amplification, and sequencing

DNA were extracted from 0.14g of stem fragments using the CTAB method (Doyle & Doyle, 1987). DNA extractions were visualized under UV light following gel electrophoresis on a 0.7% agarose gel (Fisher Scientific Midi Submarine Gel). The DNA concentration and quality were determined using spectrophotometry (NanoDrop™ Lite, Thermo Fisher Scientific Inc., Waltham, MA, USA).

The selected DNA barcodes were amplified based on a polymerase chain reaction (PCR). Amplification reactions were conducted in a total volume of 30µL, which contained 3µL of DNA extraction (10–50ng), 15µL 2x BioMix Red (Bioline™ Reagents Ltd., London, UK), 0.9µL of each primer (Sigma–Aldrich™, Schnellendorf,

Germany), and Milli-Q water to adjust the volume. The primer sequences for each barcode are summarised in Table 1. The PCR program for all makers was as follows: 97°C for 120sec; followed by 30 cycles of 97°C for 60sec, 52°C for 60sec, and 72°C for 120sec; and a final extension at 72°C for 7min. The annealing temperature was raised to 55°C with ITS internal primers of Blattner (1999). The PCR products were examined using 1% agarose gels and UV light, then purified and sequenced by the Eurofins Genomics company (Cologne, Germany).

#### Data analyses

Sequences were assembled and edited by SeqMan Pro™ (DNASTar, Inc., Madison, USA). The number of informative sites, variable sites, and average guanine-cytosine content were calculated using Molecular Evolutionary Genetics Analysis (MEGA)-X Version 10.2.4 (Kumar et al., 2018). Two methods were performed to test the effectiveness of the candidate barcodes for the molecular identification of *C. lodarensis*: a similarity-based method using the basic local alignment search tool (BLASTn) (Altschul et al., 1990) and tree-based methods.

#### BLASTn search

To evaluate the species identification ability of candidate markers, PCR products of three amplified fragments of *C. lodarensis* were queried against the nucleotide database in GenBank using a BLASTn search available at <https://blast.ncbi.nlm.nih.gov/Blast.cgi>. The criteria for successful identification were the top matching hits with the lowest E-value and the highest maximum percent identity score, as described by Ross et al. (2008). The threshold for identification success was determined by a BLASTn cutoff value of ≥ 99% identity for a top match.

TABLE 1. List of primer sequences used in the study

Region	Primer name	Primer sequences 5'-3'	Reference
<i>trnL-trnF</i>	c	CGAAATCGGTAGACGCTACG	(Taberlet et al., 1991)
	f	ATTTGAACTGGTGACACGAG	
<i>psbA-trnH</i>	psbAF	GTTATGCATGAACGTAATGCTC	(Sang et al., 1997)
	trnHR	CGCGCATGGTGGATTACAAAATC	
ITS	ITS4	TCCTCCGCTTATTGATATGC	(White et al., 1990)
	ITS5	GGAAGTAAAAGTCGTAACAAGG	
Internal primers	ITS-A	GGAAGGAGAAGTCGTAACAAGG	(Blattner, 1999)
	ITS-B	CTTTTCCTCCGCTTATTGATATG	
	ITS-C	GCAATTCACACCAAGTATCGC	
	ITS-D	CTCTCGGCAACGGATATCTCG	



### Phylogenetic analysis

Three different phylogenetic methods were used to evaluate barcode effectiveness at the species- and section-level discrimination (clustering properties). These techniques are maximum likelihood (ML), maximum parsimony (MP) and neighbour joining (NJ) under the Kimura 2-parameter distance model.

The newly generated sequences were combined with those retrieved from GenBank. Multiple sequence alignment was built for each barcode using the MUSCLE algorithm (Edgar, 2004) in AliView (Larsson, 2014). Single barcode regions were then concatenated to produce a combined dataset of all three markers. MEGA-X (Kumar et al., 2018) was used to build maximum parsimony and neighbour-joining trees. Maximum-likelihood trees were generated using Randomized Axelerated Maximum Likelihood–high-performance computing (RAXML-HPC) implemented in the CIPRES Science Gateway 3.3 (Miller et al., 2010). Bootstrap support values (BP) were computed for all analysis methods by executing 1000 replicates. Additionally, phylogenetic trees were visualised and edited using iTOL version 6.5.8 (Letunic & Bork, 2021). Positive species identification by candidate barcodes was only counted if those species accessions formed a cluster with bootstrap support exceeding 50%.

## Results

### Amplification and sequencing

Two accessions of *C. lodarensis* were sampled in this study: S18a and S6B. All regions for S18a were successfully amplified and sequenced, but S6B was excluded from the analysis due to poor sequencing quality.

The internal transcribed spacer failed to amplify in one segment using ITS4 and ITS5 primers. Instead, the internal transcribed spacer was amplified in two segments, ITS1 and ITS2, using Blattner (1999) primers, which yielded high-quality bidirectional sequences along with *psbA-trnH*. For *trnL-trnF*, this locus produced low-quality sequences with ambiguous base calls that required manual editing. The newly obtained sequences were submitted to GenBank under accession numbers MZ441365, MZ447872, and MZ447871 for ITS, *psbA-trnH*, and *trnL-trnF*, respectively.

### Characteristic analysis of DNA regions

Among the candidate DNA barcodes,

the nuclear locus ITS had the highest level of parsimony-informative sites (15.2%), polymorphisms (26.4%), and guanine–cytosine content (50.3). Meanwhile, *trnL-trnF* had the lowest values of all these parameters. Table 2 summarises the number of informative and variable sites and the average content of GC for each marker calculated with MEGA-X.

### Species identification using BLASTn

The reliability of species identifications for candidate markers was assessed using a BLASTn search. In this analysis, sequences of *C. lodarensis* (*H. lodarensis*) were queried against the online nucleotide database in NCBI. This exploration retrieved deposited sequences of *H. lodarensis* for ITS (KF677355.1) and *trnL-trnF* (KF677970.1) but no sequences for *psbA-trnH*. Based on an identity cutoff of  $\geq 99\%$ , 16 accessions of ITS matched our sample, and all belonged to *Huernia*. The highest was 99.85% between our sample and *H. keniensis*, while it was 99.55% between our sample and the earlier reported *H. lodarensis* (KF677355.1). The lowest was 99.25% with *H. hupatana*. In *psbA-trnH*, our sequences showed the highest identity percentage (100.00%) with *H. concinna*. For *trnL-trnF*, no sequences matched the threshold identity percentage, and the highest was 98.41% between our sample and *H. erectiloba*. Table 3 shows the first five related species retrieved from the BLASTn search for each locus.

### Species discrimination using tree-based methods

Positive species identification by three potential barcodes and their combination was determined by the formation of highly supported ( $>50\%$ ) monophyletic clades using the ML, MP and NJ methods. No single independent clade was formed for *H. lodarensis* for candidate barcodes or their combination in all tree-building methods (Figs. 2–5). Nuclear ITS presented the best performance among the three markers for section discrimination. In addition, the species of sect. *Huernia* was strongly supported as a monophyletic group, with bootstrap values of 85%, 86% and 79% for the ML, MP and NJ trees, respectively (Fig. 2). Using a three-gene combination (ITS+ *psbA-trnH*+ *trnL-trnF*), we obtained 100% and 98% support for the monophyly of the sect. *Huernia* clade in ML and MP analyses, respectively, but NJ analysis did not support monophyly (Fig. 5).

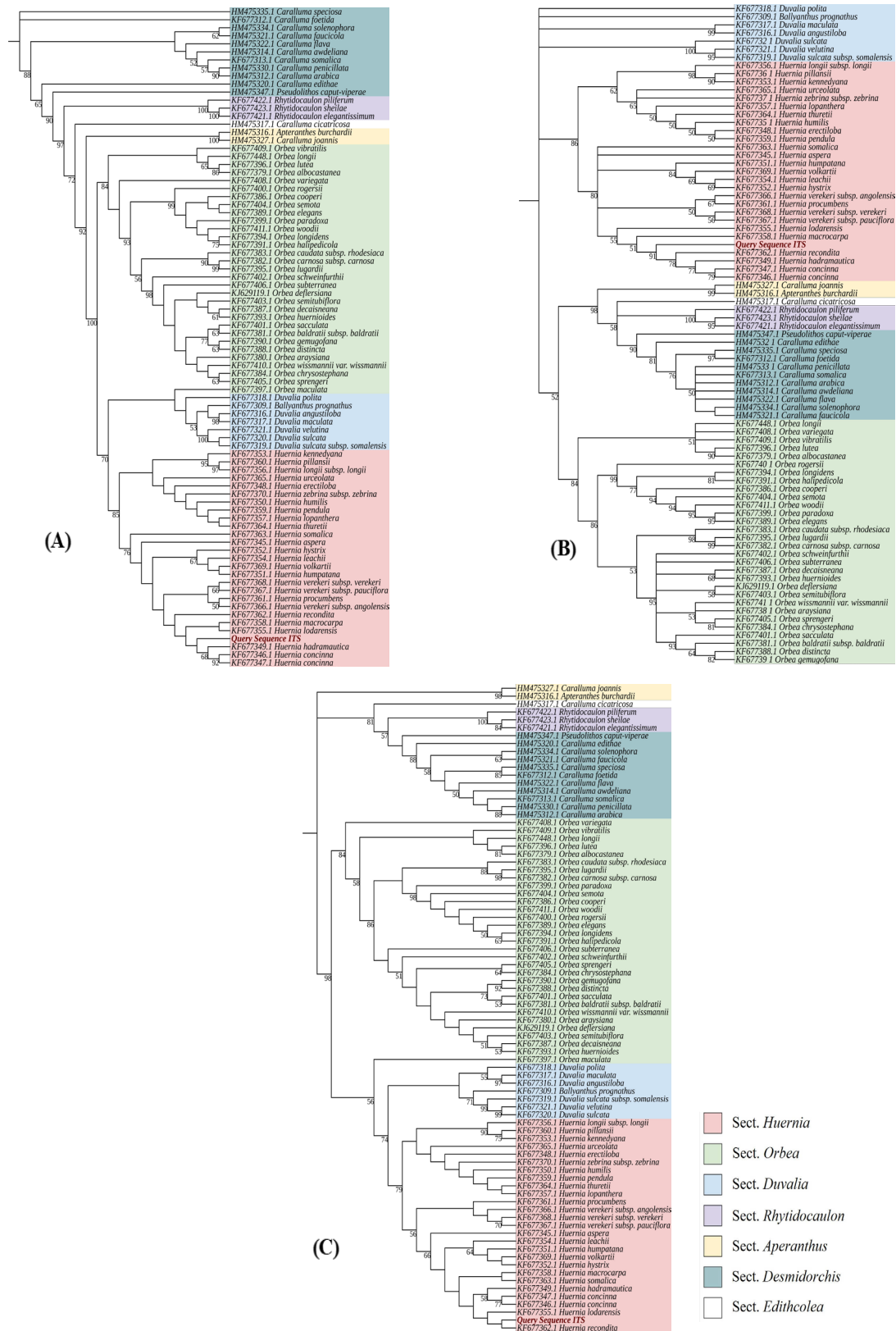


**TABLE 2. Sequence characteristics of the potential DNA barcodes ITS, *psbA-trnH*, and *trnL-trnF***

Variable	ITS	<i>psbA-trnH</i>	<i>trnL-trnF</i>
Amplicon length (bp)	745	352	754
Aligned length (bp)	685	780	909
No. of samples	82	84	82
Parsimony informative sites (%)	15.2	12.4	2.6
Variable sites (%)	26.4	22.4	6.1
Average of GC content	50.3	25	37.4

**TABLE 3. First five related species retrieved from the BLASTn search that matched with *Ceropegia lodarensis* (= *Huernia lodarensis*) from Saudi Arabia for three DNA barcodes: ITS, *psbA-trnH* and *trnL-trnF***

DNA barcode	Related species	Query cover %	E value	Identity %	Accession
ITS	<i>Huernia keniensis</i>	87	0.0	99.89	AJ488802.1
	<i>Huernia hadramautica</i>	89	0.0	99.70	KF677349.1
	<i>Huernia verekeri</i> subsp. <i>angolensis</i>	89	0.0	99.55	KF677366.1
	<i>Huernia recondita</i>	89	0.0	99.55	KF677362.1
	<i>Huernia lodarensis</i>	89	0.0	99.55	KF677355.1
<i>psbA-trnH</i>	<i>Huernia concinna</i>	83	4e-150	100.00	KF677653.1
	<i>Huernia somalica</i>	83	2e-148	99.66	KF677670.1
	<i>Huernia humpatana</i>	83	2e-148	99.66	KF677658.1
	<i>Huernia thuretii</i>	83	1e-146	99.32	KF677671.1
	<i>Huernia kennedyana</i>	83	1e-146	99.32	KF677660.1
<i>trnL-trnF</i>	<i>Huernia erectiloba</i>	100	0.0	98.41	KF677963.1
	<i>Huernia aspera</i>	100	0.0	98.41	KF677960.1
	<i>Huernia erectiloba</i>	100	0.0	98.41	AY780494.1
	<i>Huernia lodarensis</i>	100	0.0	98.28	KF677970.1
	<i>Huernia verekeri</i> subsp. <i>pauciflora</i>	100	0.0	98.28	KF677982.1



**Fig. 2. Phylogenetic trees based on the nrITS of *Ceropogia lodarensis* ( $\equiv$  *Huernia lodarensis*) and related species. [Trees built using the (A) maximum likelihood (ML), (B) maximum parsimony (MP) and (C) neighbor-joining (NJ) tree methods. Bootstrap values  $\geq$  50% are shown near nodes]**

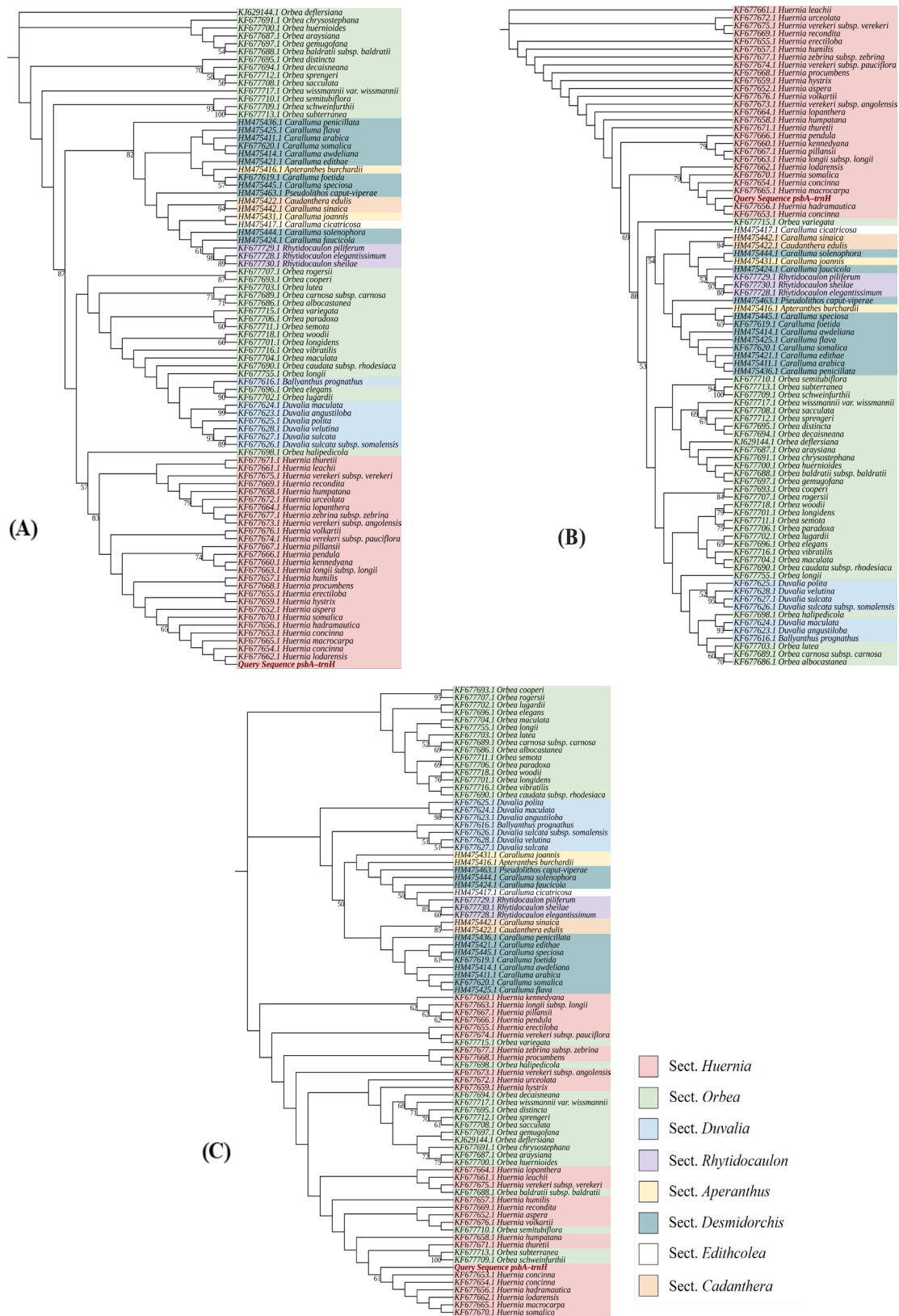


Fig. 3. Phylogenetic trees based on cp *psbA-trnH* of *Ceropogia lodarensis* (= *Huernia lodarensis*) and related species [Trees built using the (A) maximum likelihood (ML), (B) maximum parsimony (MP) and (C) neighbor-joining (NJ) tree methods. Bootstrap values  $\geq 50\%$  are shown near nodes]



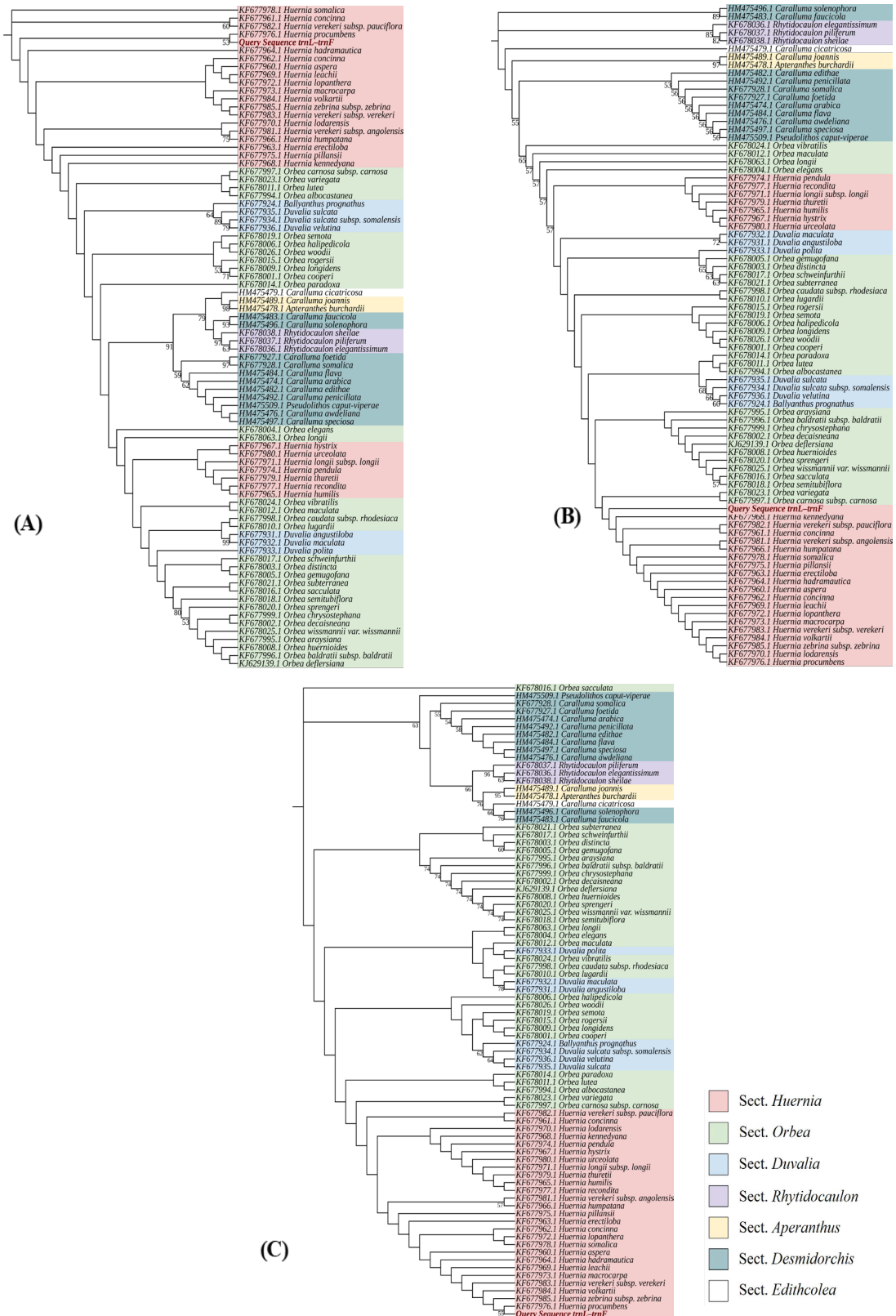


Fig. 4. Phylogenetic trees based on cp *trnL-trnF* of *Ceropogia lodarensis* ( $\equiv$  *Huernia lodarensis*) and related species [Trees built using the (A) maximum likelihood (ML), (B) maximum parsimony (MP) and (C) neighbor-joining (NJ) tree methods. Bootstrap values  $\geq 50\%$  are shown near nodes]

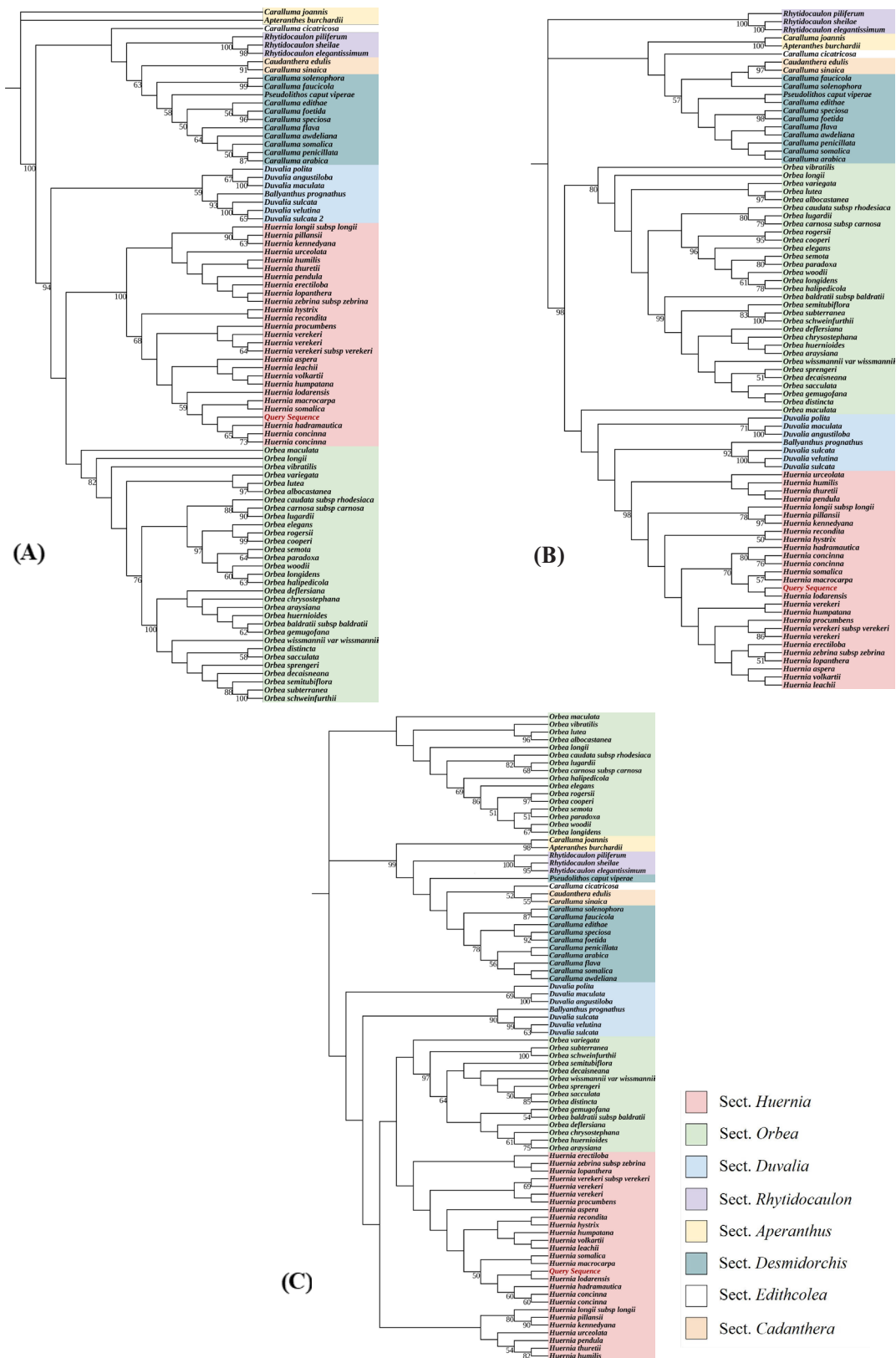


Fig. 5. Phylogenetic trees based on three-gene combination (ITS+ *psbA-trnH*+ *trnL-trnF*) of *Ceropogia lodarensis* (= *Huernia lodarensis*) and related species [Trees built using the (A) maximum likelihood (ML), (B) maximum parsimony (MP) and (C) neighbor-joining (NJ) tree methods. Bootstrap values  $\geq 50\%$  are shown near nodes]

## Discussion

Traditional medicine is a crucial part of Saudi Arabia's heritage, which is commonly practiced today (Awadh Ali et al., 2017). Saudi Arabia has a diverse flora (2288 spp.), with over 1200 species expected to have therapeutic activity (Alqahtani et al., 2014). For example, several species of sect. *Huernia* have ethnopharmacological uses in the treatment of diabetes and wound healing (El Sayed et al., 2018; Hamam et al., 2018). However, traditional identification of this plant group is difficult due to morphological similarities. Several studies have been conducted to explore the medicinal value of *Huernia* species (Ali et al., 1984; Almehdar et al., 2012; Alzahrani et al., 2015; El Sayed et al., 2018; Hamam et al., 2018; El Sayed et al., 2020) and determine their evolutionary history (Bruyns et al., 2014). However, no known studies have focused on molecular identification of the species. The numerous pharmacological properties of *C. lodarensis* and its wide habitat range in the Arabian Peninsula make this species an excellent candidate to investigate the DNA barcoding method for the molecular identification of sect. *Huernia* species.

The DNA barcoding technique has been proven to be an effective tool for distinguishing medicinal plant species (Al-Qurainy et al., 2011; Khan et al., 2017; Alkaraki et al., 2021). However, this study revealed that the nuclear ITS and the chloroplast *psbA-trnH* and *trnL-trnF* barcodes had a low capacity for identifying *C. lodarensis* at the molecular level. A BLASTn search revealed high similarities among the sect. *Huernia* sequences (Table 3). This low nucleotide sequence variability could be attributed to either the recent radiation of sect. *Huernia* species in the Arabian Peninsula and Horn of Africa (Bruyns et al., 2014), or the possible occurrence of natural hybridisation in this plant group (Harvey, 2014). DNA barcoding has been reported to be challenging in plant genera that are prone to hybridisation and have a relatively young age (Roy et al., 2010). Therefore, molecular identification of *C. lodarensis* by candidate barcodes using BLASTn search appears ineffective at the species level.

The ITS region was significantly more informative than the *psbA-trnH* and *trnL-trnF* loci, and it had the highest percentage of variable sites (26.4%), which is consistent with the rapid evolutionary rate of ITS (Li et al., 2011). The higher variability of the ITS region than plastid loci has made it widely used in plant phylogenetic

studies (Kress et al., 2005; Chase et al., 2007). In this paper, the ITS region showed the highest rate of identification success at the sect. *Huernia* level using the tree-based method.

For all methods of tree building with ITS, *C. lodarensis* was nested among a strongly supported monophyletic group of sect. *Huernia* species (BP=85%, 86% and 79% for ML, MP and NJ trees, respectively) (Fig. 2). This locus has previously demonstrated good species identification capabilities in stapeliads. *Hoodia* detection and identification were accomplished by ITS1 (Gathier et al., 2013), whereas ITS2 demonstrated high discriminatory capacity in *Caralluma*, *Boucerosia*, *Desmidorchis*, *Duvalia* and *Echidnopsis* (Selvaraj et al., 2015). Taken together, these findings suggest that the nuclear ITS is a promising DNA barcode for section-level identification of sect. *Huernia* species.

The chloroplast loci *psbA-trnH* and *trnL-trnF* were less effective at the sect. *Huernia* level. This result is consistent with previous studies finding that found low nucleotide variation of *psbA-trnH* in many genera, such as *Solidago* (Kress et al., 2005), *Lysimachia* (Zhang et al., 2012), and *Aspalathus* (Edwards et al., 2008). This limitation reduces the utility of *psbA-trnH* in sect. *Huernia* research, despite its high discrimination power at the species level in several plant groups (Sang et al., 1997; Chandler et al., 2001; Kress & Erickson, 2007; Lahaye et al., 2008). Similarly, *trnL-trnF* is the most conservative marker among the candidates (sequence similarity account for 91.30%) and had the lowest performance, despite its high discriminatory resolution for *Taxus* species identification (Liu et al., 2011, 2018). Therefore, the results indicated that the chloroplast loci *psbA-trnH* and *trnL-trnF* are not suitable DNA barcodes for the molecular identification of sect. *Huernia* species.

Combining barcodes, when compared to using a single locus, increases discriminatory power and showed clear benefits for species identification (CBOL, 2009; Hollingsworth et al., 2011; Yan et al., 2011). However, the use of a multilocus barcode was not significantly beneficial in our case. Many studies have similarly shown a lack of improvement in species discrimination when utilising DNA region combinations (Starr et al., 2009; Pettengill & Neel, 2010). An increase in the clade support for sect. *Huernia* was demonstrated with three loci barcode ITS + *psbA-trnH* + *trnL-trnF* using the ML and MP trees (BP=100% and



98%, respectively) (Fig. 5).

### **Conclusion**

This research examined the capacity of three DNA barcodes to identify the medicinal plant *C. lodarensis* ( $\equiv$  *Huernia lodarensis*) at the molecular level. The present study is one of the first attempts to investigate the potential of molecular identification in a species of *Ceropegia* sect. *Huernia*. The results showed that the nuclear ITS and plastid regions *psbA-trnH* and *trnL-trnF* have low species discrimination power. This finding could be attributed to the low nucleotide sequence variability observed among the *Ceropegia* sect. *Huernia*. However, the ITS region showed the highest rate of section-level identification success and could be used to distinguish this plant group from closely related sections of *Duvalia* and *Orbea*. Despite its exploratory nature, this study offers insight into the usefulness of DNA barcodes in distinguishing plant fragments and specimens that lack flowers of the *Ceropegia* sect. *Huernia* from their closely related stapeliads sections in the herbal medicine trade. Considerably more work should be undertaken to test further DNA loci and investigate the utility of other molecular identification tools to identify this complex and valuable plant group.

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### تقييم فعالية ثلاثة مناطق جينومية في تعريف النوع الطبي *Ceropegia lodarensis* (الفصيلة الأبوسينية) (Lavranos) Bruyns

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ينتمي نبات *Ceropegia lodarensis* إلى قسم *Huernia* التابع لجنس *Ceropegia*، وهو القسم الأكثر تنوعاً وانتشاراً بين النباتات ذات السيقان العصارية المعروفة باسم stapeliads. يتميز النوع النباتي موضع الدراسة بالعديد من الخصائص الدوائية التي جعلته احد العلاجات الشعبية لداء السكري و الجروح في الجزيرة العربية. بالرغم من ذلك، يعد تعريف هذا النبات بناءً على الصفات المظهرية صعب في معظم الحالات وقد يستحيل في الافراد الغير مزهره. لقد تغلبت تقنية DNA barcoding على كثير من التحديات التي قد تواجه طرق تعريف النباتات التقليدية، لذلك، يهدف هذا البحث إلى توظيف هذه التقنية واختبار قدرة ثلاث مناطق جينومية (ITS) (*psbA-trnH*, *trnL-trnF*) على تعريف *C. lodarensis* على المستوى الجزيئي. تم تحليل التشابه في تنابعات DNA للمناطق الجينومية المختبرة للنبات مقابل التنابعات المتاحة في GenBank باستخدام BLASTn كما تم بناء أشجار النشوء والتطور لقسم *Huernia* والاقسام وثيقة الصلة مثل *Duvalia* و *Orbea* باستخدام ثلاث طرق إحصائية (maximum parsimony، neighbor joining، maximum likelihood). بينت النتائج أن المناطق المرشحة لديها قدرة منخفضة على تمييز *C. lodarensis* عن الأنواع وثيقة الصلة في قسم *Huernia* وقد اظهر ITS أعلى معدل نجاح في تحديد هوية النبات على مستوى القسم. لازالت الحاجة ماسه لاختبار مواقع جينومية أخرى تكون ذات تباين اعلى في تنابعات DNA من المناطق المختبرة في هذا البحث.