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Quantitative morphology and *rbcL* barcode of *Leontodon tuberosus* L. (Asteraceae) from Al-Jabal Al-Akhdar, Libya: a baseline and the first Libyan GenBank *rbcL* record

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Abstract

Leontodon tuberosus L. (Asteraceae, Cichorieae) is a Mediterranean tuberous perennial, but quantitative morphological data and molecular reference sequences from Libya are lacking. We recorded 14 traits (11 measured, three derived) in 40 plants collected haphazardly at flowering in Al-Bayda, northeastern Libya, and tested generic placement using rbcL. Weight traits and organ counts varied most (coefficient of variation, CV, 31.0–47.4%); linear dimensions varied less (CV 19.8–24.6%), except flowering-stalk length (32.7%). Capitulum diameter was the most uniform trait (3.62 ± 0.04 cm, mean ± SE; CV 7.7%). Fresh and dry weights of each organ were strongly correlated (r = 0.84–0.91), whereas leaf count and root traits were moderately correlated with organ weights (r = 0.45–0.57; FDR-adjusted $P < 0.01$). Root length, leaf count and root count explained 58% of the variation in tuberous-root fresh weight. Flower count was not significantly correlated with any trait ($R^2 = 4.4\%$, P = 0.650). A 570-bp *rbcL* sequence from pooled leaf DNA (LAZA2, GenBank PZ426799) was assigned to *Leontodon*, identical to an *L. tuberosus* reference (LC911140). It differed from *L. hispidus* at one nucleotide (99.8% identity) and was 97.9–98.7% identical to 18 genera. A Neighbor-Joining tree placed it in the

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Leontodon clade (96% bootstrap). Because species separation rests on one nucleotide, *rbcL* confirms the genus only; species identity relies on diagnostic morphology and two herbarium vouchers. To our knowledge, this is the first Libyan *rbcL* sequence of *L. tuberosus* in GenBank. Future studies should sequence individual plants from several populations and add matK, trnH-psbA and ITS.
Keywords: *Leontodon tuberosus*; morphometrics; DNA barcoding; *rbcL*; Cichorieae.

التوصيف المورفومتري الكمي والباركود الجيني rbcL لنبات Leontodon tuberosus L. (Asteraceae) من الجبل الأخضر، ليبيا: بيانات مرجعية أولية وأول تسلسل rbcL ليبي مودَع في GenBank

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الملخص:

يُعدّ نبات Leontodon tuberosus L. (الفصيلة المركّبة، قبيلة Cichorieae) من المعمّرات الدرنية في حوض المتوسط، وتفتقر ليبيا إلى بيانات مورفولوجية كمية وتسلسلات جزيئية مرجعية عنه. سجّلنا 14 صفة (11 مقيسة وثلاث مشتقة) في 40 نباتاً جُمعت عشوائياً عند الإزهار في مدينة البيضاء بشمال شرق ليبيا، واختبرنا التموضع على مستوى الجنس بالباركود البلاستيدي rbcL. كانت الأوزان وأعداد الأعضاء الأكثر تبايناً (معامل الاختلاف CV 31.0–47.4%)، والأبعاد الخطية أقل (19.8–24.6%) عدا طول الحامل الزهري (32.7%). وكان قطر النورة الرأسية الأكثر تجانساً (3.62 ± 0.04 سم، المتوسط ± الخطأ المعياري؛ CV 7.7%). ارتبط الوزنان الطازج والجاف للعضو

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الواحد ارتباطاً قوياً (r = 0.84–0.91)، وارتبط عدد الأوراق وصفات الجذور ارتباطاً متوسطاً بأوزان الأعضاء (r = 0.45–0.57؛ P < 0.01 بعد تعديل معدل الاكتشاف الخاطئ). وفسّر طول الجذر وعدد الأوراق وعدد الجذور 58% من تباين الوزن الطازج للجذور الدرنية، ولم يرتبط عدد الأزهار معنوياً بأي صفة (R² = 4.4%، P = 0.650). أُسند تسلسل rbcL بطول 570 زوجاً قاعدياً من الحمض النووي المجمّع للأوراق (LAZA2، بنك الجينات PZ426799) إلى الجنس Leontodon، وطابق التسلسل المرجعي لـ L. tuberosus (LC911140)، واختلف عن L. hispidus بنيوكليوتيد واحد (تطابق 99.8%)، وتطابق بنسبة 97.9–98.7% مع 18 جنساً. ووضعته شجرة الجوار المتجاور ضمن فرع Leontodon (دعم بوتستراب 96%). ولأن الفصل بين الأنواع يستند إلى نيوكليوتيد واحد، يؤكد rbcL الجنس فقط، وتعتمد هوية النوع على الصفات المورفولوجية التشخيصية وعينتين معشبيتين مرجعيتين. وحسب علمنا، هذا أول تسلسل rbcL لـ L. tuberosus من ليبيا في بنك الجينات. وتوصي الدراسة بتسلسل نباتات فردية من عدة مجتمعات وإضافة matK وtrnH–psbA وITS لتحسين التمييز على مستوى النوع.

الكلمات المفتاحية: Leontodon tuberosus؛ القياسات المورفولوجية الكمية؛ الباركود الجيني؛ rbcL؛ Cichorieae

1. Introduction

The tribe Cichorieae (Asteraceae) comprises latex-bearing, ligulate-flowered taxa whose classification has long been hampered by homoplasy, polyploidy and morphological convergence. In the subtribe Hypochaeridinae, species of *Leontodon* L. (approximately 40–50 taxa centred in the Mediterranean basin; Moura *et al.*, 2015; Conti *et al.*, 2025a) are traditionally delimited by indumentum, achene and pappus dimorphism and capitulum architecture, characters that often overlap among close relatives (Zidorn, 2012). Molecular phylogenies showed that *Leontodon* in its traditional circumscription is diphyletic, which led to the segregation of *Scorzoneroides* (Samuel *et al.*, 2006) and to later revisions of its internal limits (Enke *et al.*, 2012). Because polymorphism,

hybridisation and allopolyploidy are frequent, morphology alone is often insufficient to resolve species limits (Samuel *et al.*, 2006; Conti *et al.*, 2025a).

Leontodon tuberosus L., the tuberous hawkbit, is a perennial, rosette-forming herb with tuberous roots, scapose stems and solitary yellow capitula. It belongs to section *Thrincia*, characterised by 2–3-fid hairs, 2n = 8 and heterocarpic achenes, and it is the only species of the section with root tubers (Samuel *et al.*, 2006; Zidorn, 2012). It occurs from southern Europe to north-western Africa, Cyprus and western Syria (Vladimirov and Dimitrova, 2006) and is a traditionally gathered wild food plant in several Mediterranean countries (Kozuharova *et al.*, 2025). In Libya, it has been recorded from the coastal plains, Jabal Nafusa and Al-Jabal Al-Akhdar (Jafri and El-Gadi, 1983; El-Mokasabi, 2014; Sherif *et al.*, 2020), but no molecular reference sequence from the country has been available. Sesquiterpenoids and phenolic constituents have been isolated from it and its congeners (Zidorn and Stuppner, 2001; Di Lecce *et al.*, 2022), which makes reliable identification a prerequisite for phytochemical and pharmacological work.

Morphological information on *L. tuberosus* is scattered across floras and keys, is mostly qualitative, and is rarely accompanied by ranges, sample sizes or statistical treatment, so the reliability of the characters used in field identification has seldom been tested quantitatively. Integrative studies combining multivariate morphometrics with molecular data have resolved closely related *Leontodon* taxa, distinguished hybrids from their parents and supported the description of a new subspecies (Moura *et al.*, 2015; Conti *et al.*, 2025b). To our knowledge, however, no study has combined a quantitative morphological characterisation of *L. tuberosus* with molecular verification.

DNA barcoding complements classical taxonomy because it is rapid, repeatable and independent of the developmental stage of the material (Hebert *et al.*, 2003). For land plants, the CBOL Plant Working Group (2009) proposed *rbcL* and *matK* as the core barcode. *rbcL* amplifies and sequences reliably, is extensively represented in public repositories and performs well for genus-level assignment, but its discrimination among closely related species is

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lower than that of *matK*, *trnH-psbA* or ITS (Li *et al.*, 2011), and its accuracy is bounded by the completeness of the reference library (Kress, 2017).

We therefore (i) documented the vegetative and reproductive organs of *L. tuberosus* photographically; (ii) quantified the variation of 14 vegetative, reproductive and biomass traits (11 measured directly and three derived) among 40 plants collected haphazardly and examined the relationships among the directly measured traits by Pearson correlation and multiple regression; and (iii) tested the generic placement of the population with the plastid *rbcL* barcode, deposited a reference sequence in GenBank (to our knowledge, the first GenBank record for the species from Libya), and evaluated how well *rbcL* separates *L. tuberosus* from its closest congener in the available reference set. The molecular analysis was designed to confirm identity and not to assess intraspecific genetic variation. The data provide a morphological and molecular baseline for taxonomic, ecological, phytochemical and conservation studies.

2. Materials and Methods

2.1 Study area and plant material

Forty individuals of *Leontodon tuberosus* L. were collected haphazardly (without a randomised design or predefined sampling points) on the campus of Omar Al-Mukhtar University, Al-Bayda (32°45′30″ N, 21°42′44″ E; elevation 606 m a.s.l.), Al-Jabal Al-Akhdar region, northeastern Libya, during April 2026. The Al-Jabal Al-Akhdar plateau is characterized by a Mediterranean-type climate (Köppen Csb), with mean annual precipitation of approximately 540 mm concentrated mainly in the autumn and winter months, elevations reaching up to c. 880 m above sea level, and calcareous, karstic soils supporting maquis, garrigue, and steppe-like vegetation (Al-Sodany *et al.*, 2003). This region constitutes the only substantial Mediterranean forest and semi-natural vegetation zone in Libya and hosts a notable proportion of the country's native and endemic flora. The plants were collected at the flowering stage and were selected haphazardly within the campus, without predefined sampling sites or a randomisation scheme, so that the natural range of phenotypic variation of the local population would be represented; soil and

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environmental variables were not measured. Whole plants, including intact root systems, were carefully excavated using a hand trowel to avoid damage to the tuberous roots, transported to the laboratory in labelled plastic bags, and processed within 24 h of collection. Plants were provisionally identified in the field from diagnostic vegetative and floral characters described in the regional floras (Kupicha, 1975; Ali *et al.*, 1976–1989), and their generic identity was subsequently checked with the *rbcL* DNA barcode (sections 2.3–2.6). After all measurements had been completed, two of the 40 plants were retained as dried voucher specimens and deposited in the Silphium Herbarium, Department of Botany, Faculty of Science, Omar Al-Mukhtar University, under registration number 135.83.3.7; these plants also contributed leaves to the pooled DNA sample (five leaves from each of the two voucher plants, i.e. 10 of the 200 leaves; section 2.3). The code LAZA2 is the sample ID recorded in the GenBank entry and is not a herbarium voucher number, so the sequence is linked to the voucher specimens through the pooled sample and not through a voucher field of the GenBank record.

2.2 Morphological characterization

For each of the sampled individuals, seven morphometric traits were recorded on fresh material: number of leaves per plant (LC), mean leaf length (cm; MLL), number of swollen tuberous roots per plant (RC), mean length of the tuberous roots (cm; RL), number of flower heads (capitula) per plant (FC), mean flowering-stalk length (scape plus capitulum) (cm; FSL) and mean capitulum diameter (cm; CD). Lengths and diameters were measured using a graduated ruler and a digital caliper (precision 0.001 cm). Fresh weight of the shoot (aerial) system (FWS) and of the root system, i.e. the swollen tuberous roots (FWR), were recorded separately for each individual immediately after collection using an analytical balance (precision 0.001 g). The same plant fractions were then dried at 60 °C to constant weight, a temperature chosen to protect the DNA from degradation before the later extraction, to determine shoot (DWS) and root (DWR) dry weight. These measurements gave eleven directly measured traits (seven morphometric and four weight traits). Total fresh weight (TFW), total dry weight (TDW) and dry-

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matter percentage (DMP) were then derived from these measurements, giving fourteen traits in total. All measurements were taken on the whole individual plant rather than on a subsample of individuals, and traits were recorded in the same sequence for all specimens to minimize handling-related bias. Leaf length, tuberous-root length and flowering-stalk length (scape plus capitulum) were measured on every leaf, tuberous root and capitulum of each plant and averaged per plant, and capitulum diameter was measured on five capitula per plant: the diameter of each of the five capitula was measured, the five readings were averaged, and this per-plant mean was entered into the statistical analysis as the value of that plant. Five capitula was the minimum number borne by any of the 40 plants (section 3.2); five capitula were therefore measured on every plant, so that no plant was excluded and none lacked sufficient capitula. Representative plants and organs of the studied specimens were photographed by the authors for morphological documentation (Fig. 1).

2.3 DNA extraction

After the plants had been dried and all morphological measurements completed, five healthy leaves were taken from each of the 40 sampled plants (200 leaves in total, including 10 from the two voucher plants) and pooled into a single composite sample representing the population, and genomic DNA was extracted from this pooled dried material. A composite sample was used deliberately: the aim of the molecular analysis was genus-level confirmation of the taxonomic identity of the sampled population, not the assessment of genetic diversity within it (population genetics). Individuals were therefore not sequenced separately, and the data do not address within-population haplotype diversity. Genomic DNA was extracted from the dried leaf tissue with the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Briefly, 100 mg of tissue was homogenized with a tungsten carbide bead in 400 µl of Buffer AP1 and 4 µl of RNase A (100 mg/ml) in a 2-ml safe-lock tube, and disrupted in a TissueLyser in two cycles of 1–2 min at 20–30 Hz. The lysate was incubated for 10 min at 65 °C with intermittent inversion, mixed with 130 µl of Buffer P3 and kept on ice for 5 min.

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After centrifugation (5 min, 14,000 rpm), the lysate was passed through a QIAshredder spin column (2 min, 14,000 rpm). The cleared flow-through was transferred to a fresh tube, avoiding the debris pellet, and loaded onto a silica-membrane spin column. Following the manufacturer's wash steps, DNA was eluted in 50 µl of elution buffer.

2.4 PCR amplification and gel analysis

The plastid *rbcL* barcode region was amplified with the universal primer pair rbcLa-F (5′-ATGTCACCACAAACAGAGACTAAAGC-3′) and rbcLa-R (5′-GTAAAATCAAGTCCACCRCG-3′) (Kress and Erickson, 2007; Sundari and Papuangan, 2019), synthesized by Metabion (Planegg, Germany). Each 25-µl reaction contained 12.5 µl of EmeraldAmp Max PCR Master Mix (Takara, Japan), 1 µl of each primer (20 pmol), 5.5 µl of nuclease-free water and 5 µl of template DNA. Amplification was performed in an Applied Biosystems 2720 thermal cycler under the conditions given in Table 1.

Table 1. Primers, expected amplicon size and cycling conditions used for *rbcL* amplification.

Parameter	Value
Forward primer (rbcLa-F), 5′–3′	ATGTCACCACAAACAGAGACTAAAGC
Reverse primer (rbcLa-R), 5′–3′	GTAAAATCAAGTCCACCRCG
Expected amplicon size	~600 bp
Initial denaturation	94 °C, 5 min
Amplification (35 cycles)	94 °C for 30 s; 55 °C for 40 s; 72 °C for 45 s
Final extension	72 °C, 10 min

Amplicons were resolved on a 1.5% agarose gel (AppliChem, Darmstadt, Germany) in 1× TBE at 5 V/cm at room temperature. Fifteen microliters of each product were loaded alongside a GeneRuler 100-bp DNA ladder (Fermentas, Germany) (lane L), together with a positive control (lane P; used to verify that the reaction worked), a negative control (lane N; used to verify the absence of contamination in the reagents) and the amplified leaf-DNA sample (lane S; Fig. 2), and gels were documented with an Alpha Innotech (Biometra) gel documentation system, and the images were analyzed with the accompanying software.

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2.5 Sequencing and sequence assembly

PCR products were purified with the QIAquick PCR Purification Kit (Qiagen, Valencia, CA, USA). Sequencing followed the Sanger dideoxy (chain-termination) method. Cycle sequencing used the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems), and reaction products were cleaned with Centri-Sep spin columns before capillary electrophoresis on an Applied Biosystems 3130 Genetic Analyzer. Chromatograms were inspected, edited and assembled in Lasergene DNASTAR v12.1. The final sequence was deposited in GenBank under accession PZ426799 (sample ID LAZA2; partial coding sequence of 570 bp; submitted 15 May 2026).

2.6 Sequence identity and phylogenetic analysis

The LAZA2 sequence was compared with the GenBank nucleotide database using BLAST (Altschul *et al.*, 1990) to establish its taxonomic assignment and to retrieve homologous reference accessions. The final dataset comprised 41 *rbcL* sequences: the LAZA2 sequence, the authenticated *L. tuberosus* reference LC911140 (isolate AlexBA-C8), two *L. hispidus* accessions (OR990525 and OZ251152), accessions of eight other Cichorieae genera (*Lactuca*, *Cichorium*, *Chaetoseris*, *Avellara*, *Notoseris*, *Sonchus*, *Taraxacum* and *Crepidiastrum*), and representatives of other Asteraceae tribes included as outgroups (Supplementary Table S1). Pairwise identity and divergence were calculated in Lasergene DNASTAR v12.1 for LAZA2 and 20 representative accessions (Fig. 4). Sequences were aligned with ClustalW (Thompson *et al.*, 1994; Fig. 3). Phylogenetic relationships were inferred by the Neighbor-Joining method (Saitou and Nei, 1987) using the Maximum Composite Likelihood substitution model (Tamura *et al.*, 2004) in MEGA (Tamura *et al.*, 2013). Node support was estimated from 1000 bootstrap replicates (Felsenstein, 1985; Fig. 5). Following common practice, bootstrap values above 70% were treated as reasonable and values above 90% as strong support (Hillis and Bull, 1993). To check whether records of *L. tuberosus* from Libya already existed, the GenBank nucleotide database was searched in April 2026 with the species name and the country name; no earlier record of the species from Libya was found. The BOLD

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Systems database and other repositories were not searched, so the statement that this is a first record refers to GenBank only.

2.7 Statistical analysis

All analyses were performed on the 40 plants in Minitab 21.1 (Minitab, LLC, 2021). For each of the 14 traits (11 measured directly; total fresh weight, total dry weight and dry-matter percentage derived from the shoot and root weights), the mean, standard error (SE), standard deviation (SD), coefficient of variation (CV = SD/mean × 100) and range were calculated; values are reported as mean ± SE (n = 40). For traits measured on several organs per plant, the per-plant mean was used, so each observation represents one plant. Normality was assessed with the Anderson–Darling test. Pearson correlations were calculated among the 11 directly measured traits (two-tailed, $\alpha = 0.05$), with Spearman rank correlations as a sensitivity analysis, and the 55 P values were adjusted by the Benjamini–Hochberg false-discovery-rate procedure ($q < 0.05$; Benjamini and Hochberg, 1995). The derived variables were excluded from correlation analysis because they are arithmetic functions of the shoot and root weights.

Two exploratory multiple linear regressions were fitted by ordinary least squares, with predictors chosen on biological grounds: tuberous-root fresh weight (FWR) on root count (RC), root length (RL) and leaf count (LC) (Model 1), and flower count (FC) on LC, mean leaf length (MLL) and RL (Model 2). Models were evaluated using R^2, adjusted and predicted R^2, the standard error of the regression (S) and the overall F-test. Multicollinearity was assessed with variance inflation factors (VIF < 5 accepted), unusual observations were flagged by absolute standardised residuals > 2, and assumptions were checked with residual plots and the Anderson–Darling test. Robustness was verified by refitting each model without large-residual observations, by log10 transformation of FWR (Model 1) and by Poisson regression of flower count (Model 2). The repeatability of capitulum diameter was estimated from the five readings per plant by one-way random-effects analysis of variance (plant as random factor), with intraclass correlation coefficients ICC(1,1) and ICC(1,5) and 95% confidence intervals (Shrout and Fleiss, 1979).

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3. Results and Discussion

3.1 Morphological characters and comparison with published descriptions

The sampled plants were perennial, rosette-forming herbs with a cluster of swollen, spindle-shaped, brown tubers (Fig. 1A,B). The deeply lobed, oblanceolate leaves, crowded at the crown on reddish-purple petioles, had sharply toothed margins with backward-pointing segments, a nearly glabrous upper surface, and a bristly lower surface, midrib and petioles (Fig. 1C–E). Slender scapes each bore a solitary capitulum of yellow ligulate florets only, with a hairy involucre and a greenish abaxial stripe on the outer ligules (Fig. 1F,G). Fruiting heads were globose with a white pappus, and the inner achene was slender, dark and beaked, with a plumose pappus (Fig. 1H,I). Hair forking and achene dimensions were not examined. These characters agree with the diagnostic features given for *L. tuberosus* in regional floras, in treatments of section *Thrincia* (Tutin *et al.*, 1976; Samuel *et al.*, 2006; Zidorn, 2012) and in the Libyan flora platform Edama (Edama Libya Nature Platform, n.d.). The most decisive character is the tuberous root system, which is unique to the species within the section and is used at the first couplet of identification keys (Zidorn, 2012; Vladimirov and Dimitrova, 2006). The rosette of lobed leaves with solitary capitula on simple scapes is typical of the section (Ali *et al.*, 1976–1989; Samuel *et al.*, 2006), and the backward-pointing leaf segments correspond to its runcinate-pinnatifid leaf form (Vladimirov and Dimitrova, 2006; Edama Libya Nature Platform, n.d.).

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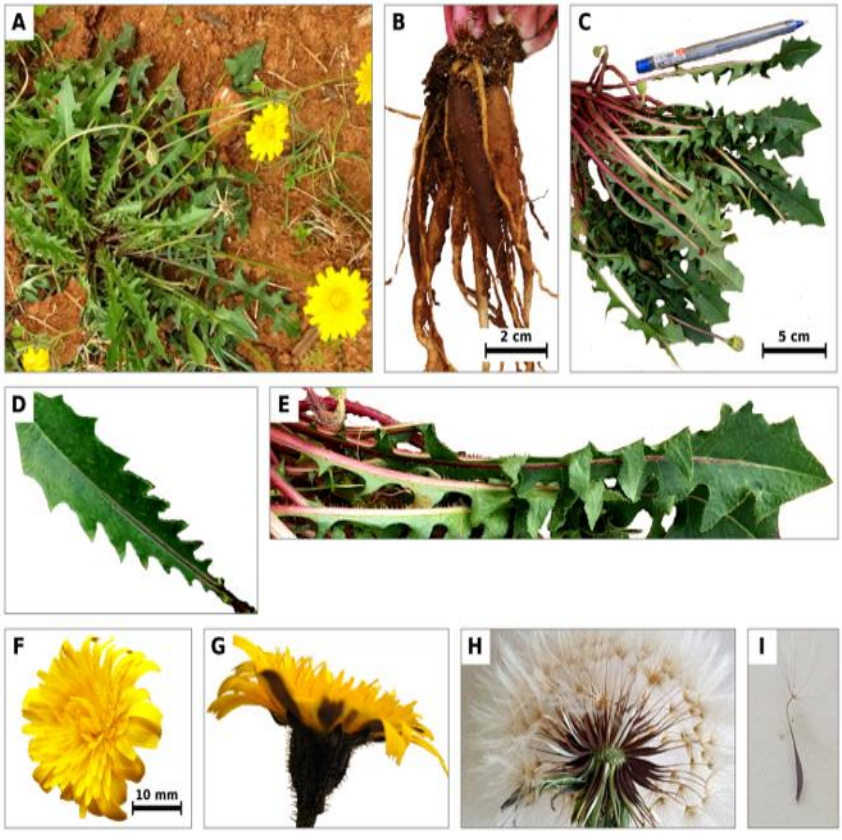


Fig. 1. Morphological characters of *Leontodon tuberosus* L. (A) whole plant in the field; (B) root system; (C) vegetative shoot with basal leaves; (D) leaf, adaxial surface; (E) leaves, abaxial surface; (F) capitulum, top view; (G) capitulum, lateral view; (H) fruiting head; (I) achene with pappus. All photographs were taken by the authors of the plants sampled in this study. Scale bars: B, 2 cm; C, 5 cm; F, 1 cm. Panels A, D, E, G, H and I have no scale bar.

Table 2 compares the traits recorded here with the Edama taxonomic description and with a horticultural guide (GardenWorld, n.d.), which is used only as supplementary information on growth habit.

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Table 2. Comparison of the morphology and growth habit of *Leontodon tuberosus* recorded in this study with the taxonomic description in Edama (Libyan flora platform) and the horticultural account in GardenWorld.

Trait	This study (n = 40)	Edama (taxonomic)	GardenWorld (horticultural)
Growth form	Rosette-forming perennial with tuberous roots	Perennial with long, slender tubers	Low herb with a basal rosette; frost-tolerant
Tubers	Swollen, spindle-shaped, brown; 6–20 roots per plant	Long, slender	Small, rounded, brown swellings on the roots that store nutrients
Leaves	8.0–25.1 cm long (mean 15.7); oblanceolate, sharply toothed to lobed, segments pointing backwards	2.0–14.0 × 0.5–2.5 cm; obovate to oblanceolate-oblong; retrorse-dentate to runcinate-pinnatifid	10–20 cm; dark green, rough, wavy margin
Stems / scapes	7.8–37.4 cm (mean 20.9), including the capitulum; 5–13 per plant	7–35 cm; 1–6 stems, simple	20–40 cm, strong, unbranched
Capitulum	Solitary; 3.01–4.20 cm in diameter (open head; plant means)	Solitary; involucre 0.9–1.5 × 0.6–1.0 cm	Solitary; “disc” about 1.5–2 cm; about 100–200 florets
Ligules	Yellow; outer ones with a greenish abaxial stripe	Yellow; outer ones with a green abaxial stripe	Yellow
Achenes	Inner morph with plumose pappus (Fig. 1I); outer morph not examined	0.3–0.7 cm; outer curved, pappus ≤ 0.05 cm; inner beaked, pappus of two rows of plumose hairs	White pappus; wind-dispersed
Flowering	April (collection date)	Not given	April–June (south); May–July (temperate climates)

Sources: Edama Libya Nature Platform (n.d.); GardenWorld (n.d.). GardenWorld is a horticultural guide and not a taxonomic treatment; “not given” means that the source does not report the trait.

Tuber shape is described differently among sources: Edama gives long, slender tubers (Edama Libya Nature Platform, n.d.), the horticultural guide small, rounded swellings (GardenWorld, n.d.), and the plants studied here had swollen, fusiform tubers, although all agree on the tuberous habit. The near-glabrous upper leaf surface, bristly lower surface and hairy involucre are compatible with the

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Edama description of rigid, 2–3-fid hairs, but hair forking could not be resolved in the photographs (cf. Samuel *et al.*, 2006). The achene in Fig. 1I is the inner morph of the heterocarpic fruit system of the section; the curved outer achenes with a reduced pappus (Samuel *et al.*, 2006; Zidorn, 2012) were not examined, and a full characterization of the fruit would require both morphs. Together, these characters are consistent with the field identification of the plants. Because *rbcL* does not separate *L. tuberosus* from *L. hispidus* with certainty (section 3.7), species identity rests on this diagnostic morphology, which can be re-examined on the two voucher specimens deposited in the Silphium Herbarium (no. 135.83.3.7).

3.2 Quantitative variation among plants

The 14 traits are summarized in Table 3 (mean ± SE, n = 40). Weight traits and organ counts varied most among plants (CV 31.0–47.4%; highest for root dry weight, 2.14 ± 0.16 g, CV = 47.4%), and linear dimensions less (root length, 11.60 ± 0.36 cm, CV = 19.8%; mean leaf length, 15.69 ± 0.61 cm, CV = 24.6%), except flowering-stalk length (20.91 ± 1.08 cm; CV = 32.7%). Capitulum diameter was the most uniform trait (3.62 ± 0.04 cm; CV = 7.7%). A plant bore on average 30.23 ± 1.67 leaves, 11.53 ± 0.61 tuberous roots and 7.58 ± 0.37 capitula, and its dry-matter percentage averaged 17.84 ± 0.51% (CV = 18.0%).

Capitulum diameter was measured on five capitula per plant, the minimum number borne by any plant (200 capitula; individual values 2.61–4.98 cm). The CV of single capitula (12.1%; mean within-plant CV 9.8%) exceeded that of the plant means (7.7%). The intraclass correlation for a single capitulum was low, ICC(1,1) = 0.25 (95% CI 0.12–0.41; $F_{39,160} = 2.64$, $P < 0.001$), so about three quarters of the variance lay among capitula within plants; the mean of five capitula had moderate repeatability (Koo and Li, 2016), ICC(1,5) = 0.62 (95% CI 0.40–0.78), with a wide interval.

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Table 3. Descriptive statistics of the 14 morphological and biomass traits of *Leontodon tuberosus* (N = 40).

Trait	Mean ± SE	SD	CV	Min–Max
Fresh weight of shoot, FWS (g)	28.64 ± 1.73	10.93	38.2%	12.48–53.36
Dry weight of shoot, DWS (g)	4.13 ± 0.22	1.42	34.4%	2.13–7.39
Fresh weight of tuberous roots, FWR (g)	7.48 ± 0.50	3.18	42.5%	2.99–14.29
Dry weight of tuberous roots, DWR (g)	2.14 ± 0.16	1.01	47.4%	0.63–4.58
Total fresh weight, TFW (g)	36.12 ± 1.86	11.73	32.5%	16.09–57.92
Total dry weight, TDW (g)	6.26 ± 0.31	1.94	31.0%	3.14–11.31
Dry-matter percentage, DMP (%)	17.84 ± 0.51	3.21	18.0%	10.32–24.83
Tuberous-root count, RC	11.53 ± 0.61	3.86	33.5%	6–20
Mean tuberous-root length, RL (cm)	11.60 ± 0.36	2.30	19.8%	8–16
Leaf count, LC	30.23 ± 1.67	10.56	34.9%	14–56
Mean leaf length, MLL (cm)	15.69 ± 0.61	3.86	24.6%	8.00–25.06
Flower count, FC	7.58 ± 0.37	2.35	31.1%	5–13
Mean flowering-stalk length (scape plus capitulum), FSL (cm)	20.91 ± 1.08	6.83	32.7%	7.78–37.35
Mean capitulum diameter, CD (cm)	3.62 ± 0.04	0.28	7.7%	3.01–4.20

Values are mean ± SE (standard error of the mean) for 40 plants; SD, standard deviation; CV, coefficient of variation (SD/mean × 100); Min–Max, minimum and maximum. TFW = FWS + FWR; TDW = DWS + DWR; DMP = TDW/TFW × 100. Leaf length, flowering-stalk length and capitulum diameter are plant means of all leaves, all flowering stalks (scape plus capitulum) and the diameters of five capitula, respectively; flowering-stalk length includes the capitulum. Root length is the mean length of the tuberous roots of each plant. Root traits (RC, RL, FWR and DWR) refer to the swollen tuberous roots (tubers). DMP was calculated for each plant and then averaged; its mean therefore differs from the ratio of mean TDW to mean TFW.

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The gradient of variability (Table 3) is the pattern expected between traits that integrate cumulative resource acquisition, such as weights and organ counts, and structurally constrained linear dimensions (Pérez-Harguindeguy *et al.*, 2013; Violle *et al.*, 2007); the low variation of capitulum diameter agrees with the relative constancy of individual reproductive units compared with total reproductive output (Weiner, 2004). Two caveats apply: dimensions averaged over several organs have a lower variance than single measurements, and weights scale approximately with the cube of linear dimensions, so higher CVs are expected for weights. Within this sample, capitulum diameter and, to a lesser extent, root and leaf length therefore appear more dependable for field identification than size-dependent traits, in line with taxonomic practice in *Leontodon* (Conti *et al.*, 2025a). Because the CV of single capitula (12.1%) was still lower than that of every other trait but ICC(1,5) was only moderate, diameter should be recorded on several capitula per plant and its diagnostic value confirmed in other populations.

Published quantitative ranges for *L. tuberosus* are scarce and come from sources of different kinds (Table 2). The mean leaf length recorded here (15.69 cm) exceeds the upper limit of the Edama range (2–14 cm) but lies within the horticultural range (10–20 cm), and the mean of 7.6 capitula per plant (range 5–13) exceeds the Edama range of 1–6 stems, each bearing one capitulum (Edama Libya Nature Platform, n.d.; GardenWorld, n.d.). Possible explanations, none testable here, include differences in how leaf length was measured (e.g. whether the petiole was included), local conditions and the sampling of well-developed plants. The flowering-stalk length (20.91 cm), which includes the capitulum, falls within both published ranges, whereas capitulum diameter (3.01–4.20 cm) could not be compared because the sources report only involucre or head “disc” dimensions. The data are observational and come from a single locality and date, so the variation cannot be partitioned into genetic, environmental and ontogenetic components and probably includes phenotypic plasticity (Violle *et al.*, 2012) and plant age. Table 3 should therefore be read as a baseline for the sampled population and not as species-wide parameters.

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3.3 Relationships among traits

Pearson correlations among the eleven directly measured traits are given in Table 4. The strongest were between the fresh and dry weights of the same organ (shoot, r = 0.838; root, r = 0.906; both P < 0.01). Leaf count correlated positively with DWS (r = 0.547), FWR (r = 0.516), DWR (r = 0.490) and FWS (r = 0.463; all P < 0.01), root length with FWR (r = 0.573) and DWR (r = 0.469; both P < 0.01), and root count with FWR (r = 0.453, P < 0.01) and DWR (r = 0.350, P < 0.05). Mean leaf length and flowering-stalk length were also correlated (r = 0.454, P < 0.01). Flower count was not significantly correlated with any trait (r = −0.183 to 0.275), and shoot weights were not significantly associated with root traits. After Benjamini–Hochberg adjustment, the ten correlations significant at P < 0.01 remained significant (q ≤ 0.018), whereas the five significant only at P < 0.05 (FWS–MLL, FWS–FSL, DWS–CD, DWR–RC and LC–CD) did not (q = 0.105–0.126) and are tentative. Five traits departed slightly from normality (Anderson–Darling P < 0.05; skewness 0.64–0.83), but Spearman rank correlations gave the same result: the same 15 pairs were significant at P < 0.05, and nine of the ten strongest remained significant after adjustment (q < 0.05), the tenth (MLL–FSL) being borderline (q = 0.051).

Table 4. Pearson correlation coefficients among the directly measured traits of *Leontodon tuberosus* (n = 40).

	FWS	DWS	FWR	DWR	RC	RL	LC	FC	MLL	FSL
DWS	0.838**									
FWR	0.117	0.209								
DWR	0.148	0.251	0.906**							
RC	0.041	0.011	0.453**	0.350*						
RL	−0.010	0.048	0.573**	0.469**	0.169					
LC	0.463**	0.547**	0.516**	0.490**	0.273	0.169				
FC	0.275	0.195	−0.067	−0.102	0.003	0.082	0.198			
MLL	0.352*	0.141	−0.234	−0.113	−0.062	−0.056	−0.277	−0.008		
FSL	0.363*	0.091	−0.216	−0.092	0.047	−0.192	−0.215	0.204	0.454**	
CD	0.235	0.352*	0.144	0.223	0.039	0.134	0.335*	−0.183	0.064	−0.128

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* Significant at $P < 0.05$; ** significant at $P < 0.01$ (two-tailed). Only the lower triangle of the matrix is shown. All coefficients marked ** remained significant after Benjamini–Hochberg FDR adjustment ($q \leq 0.018$); those marked * did not ($q = 0.105–0.126$). Trait abbreviations as in Table 3.

Plant size appears to be the main axis of covariation in this population: larger plants carry more of every organ, and allocation among leaves, stems and roots depends on size as well as on environment and ancestry (Poorter *et al.*, 2012; Weiner, 2004). Leaf count was the vegetative trait most consistently associated with biomass, and root length and count were associated with tuberous-root weight. In contrast, shoot weights were not significantly correlated with root weights or root traits (all $|r| \leq 0.26$), so the aerial and underground components varied largely independently, apart from their shared association with leaf number. This contrasts with the isometric scaling of rhizome and aboveground biomass reported across 20 perennial herbs (Harris *et al.*, 2025). A possible explanation, which the present data cannot test, is that the tubers of different plants had accumulated reserves over different numbers of seasons, so that tuber mass reflects plant age and storage history more than current shoot size; plant age was not recorded. Leaf count and mean leaf length were negatively but not significantly correlated ($r = -0.28$, $P = 0.084$); with $n = 40$, significance at $P < 0.05$ requires $|r| \geq 0.31$ and 80% power only $|r| \approx 0.43$, so a leaf size–number trade-off can be neither confirmed nor excluded.

3.4 Regression models of root biomass and flower production

Model 1: tuberous-root fresh weight. A multiple regression was fitted with tuberous-root fresh weight (FWR) as the dependent variable and RC, RL and LC as predictors (Table 5):

$$FWR = -5.90 + 0.227\ RC + 0.644\ RL + 0.109\ LC$$

The model was significant ($F_{3,36} = 16.46$, $P < 0.001$) and explained 57.84% of the variation in tuberous-root fresh weight (adjusted $R^2 = 54.32\%$, predicted $R^2 = 49.38\%$, $S = 2.15$ g). Root length ($P < 0.001$), leaf count ($P = 0.003$) and root count ($P = 0.020$) each had a significant positive effect, corresponding to 0.64 g per additional centimeter of mean root length, 0.11 g per leaf and 0.23 g per tuberous root. Collinearity was not a concern (VIF = 1.05–1.10).

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The results were robust to the exclusion of two large-residual observations (nos. 6 and 38; $R^2 = 63.03\%$; all predictors $P \leq 0.004$) and to a log10 transformation of FWR ($R^2 = 57.38\%$; all predictors $P \leq 0.013$), and the residuals met the model assumptions (Anderson–Darling $P = 0.80$). These results support the size-based interpretation above: leaf count remained associated with tuberous-root weight after accounting for root length and root count.

Model 2: flower count. A multiple regression was fitted with flower count (FC) as the dependent variable and LC, MLL and RL as predictors (Table 5):

$$FC = 5.12 + 0.0453\ LC + 0.0309\ MLL + 0.0514\ RL$$

The overall model was not significant ($F_{3,36} = 0.55$, $P = 0.650$) and explained only 4.40% of the variation in flower count (adjusted $R^2 = 0.00\%$, predicted $R^2 = -26.6\%$, $S = 2.39$); none of the predictors was significant (LC, $P = 0.244$; MLL, $P = 0.766$; RL, $P = 0.763$), and VIF values were 1.03–1.11. The result was unchanged after excluding two large-residual observations (nos. 2 and 13; $R^2 = 4.03\%$, $P = 0.701$) and was insensitive to the slight right-skew of the residuals (Anderson–Darling $P = 0.045$): Spearman correlations of flower count with LC, MLL and RL were weak and non-significant ($\rho = 0.15$, -0.09 and 0.09; $P = 0.354$, 0.581 and 0.570), and a Poisson regression gave the same result (likelihood-ratio $\chi^2 = 1.25$, 3 d.f., $P = 0.742$; Pearson χ^2/d.f. = 0.76). These results agree with the correlation analysis (Table 4).

Table 5. Multiple regression of tuberous-root fresh weight (Model 1) and flower count (Model 2).

Model	Predictor	Coefficient	SE	t	P	VIF
1	Constant	−5.90	1.99	−2.96	0.005	–
	Root count (RC)	0.227	0.0933	2.43	0.020	1.10
	Root length (RL)	0.644	0.153	4.20	<0.001	1.05
	Leaf count (LC)	0.109	0.0341	3.19	0.003	1.10
2	Constant	5.12	2.88	1.78	0.084	–
	Leaf count (LC)	0.0453	0.0383	1.19	0.244	1.11
	Mean leaf length (MLL)	0.0309	0.103	0.30	0.766	1.08
	Root length (RL)	0.0514	0.169	0.30	0.763	1.03

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Model 1: R^2 = 57.84%, adjusted R^2 = 54.32%, S = 2.15, F(3,36) = 16.46, P < 0.001. Model 2: R^2 = 4.40%, adjusted R^2 = 0.00% (Minitab displays negative adjusted and predicted R^2 values as 0.00%; the actual predicted R^2 of Model 2 was −26.6%), S = 2.39, F(3,36) = 0.55, P = 0.650. SE, standard error of the coefficient; VIF, variance inflation factor. Trait abbreviations as in Table 3.

Flower count was the trait least related to plant size. Reproductive output is often, but not always, associated with plant size (Younginger *et al.*, 2017), floral traits form a dimension largely independent of the vegetative leaf economic spectrum (E-Vojtkó *et al.*, 2022), and in *Taraxacum officinale* most of the variance in inflorescence number lay within genotypes and was attributed to phenotypic plasticity (De Giorgi *et al.*, 2024). Capitulum number in *L. tuberosus* may therefore depend more on local conditions, such as resource supply, microsite and plant age, which were not measured here, than on the vegetative traits examined. In addition, only flowering plants were sampled and none had fewer than five capitula (12 of the 40 had exactly five), so the narrow range of the trait (5–13) limited the power to detect relationships and size thresholds for flowering could not be examined. All relationships reported here are associations within a single sample and do not establish causation.

3.5 Distribution, phenology and ecological context

Leontodon tuberosus is treated as wild in Libya by the Edama platform (life-form code G, Mediterranean phytochorion), with records from the Zliten–Misurata and Benghazi plains, Jabal Nafusa and Al-Jabal Al-Akhdar (Edama Libya Nature Platform, n.d.). The Flora of Libya and later surveys document it in the same regions, including the Msallata–Garaboulli Province (Sherif *et al.*, 2020) and, in Al-Jabal Al-Akhdar, Wadi Al-Kuf (El-Mokasabi, 2014), Wadi Haboon (El-Rabiai and Al tira, 2015), the Slouk–Al-Abiar semi-desert (Raheel *et al.*, 2016) and Wadi Derna (Abu-Aziza *et al.*, 2017). The Flora of Libya gives its flowering period as February to April (Jafri and El-Gadi, 1983); flowering is reported from October to June in Italy (Portal to the Flora of Italy, n.d.) and from April to June in southern regions (GardenWorld, n.d.), which agrees with the flowering plants collected here in April. World Flora Online accepts the name, includes Libya in its native range and lists synonyms

based on Libyan or neighbouring material, such as *Thrincia tripolitana* and *L. tuberosus* subsp. oliverii (WFO, n.d.). The present record is therefore a new local record of a documented taxon and not a new country record, and the infraspecific rank of the sampled plants was not assessed. The horticultural account describes a sun-loving plant of dry, well-drained sandy to stony soils (GardenWorld, n.d.), which agrees with the calcareous, karstic soils and open vegetation of Al-Jabal Al-Akhdar (section 2.1), although light and soil conditions were not measured at the sampling site.

Leontodon tuberosus is native to Libya and across the Mediterranean basin (POWO, n.d.), and wild Asteraceae are among the most frequently consumed wild food plants of the region (Leonti *et al.*, 2006; Kozuharova *et al.*, 2025). The southern and eastern Mediterranean is documented far less than the north (Egypt, Libya and Syria each contributed less than 1% of recorded wild edible plant uses; Gori *et al.*, 2026), and the first ethnobotanical survey of edible wild plants in Libya recorded 145 species, none of them *L. tuberosus* (Mahklouf, 2019). Morphometric baselines such as those presented here are therefore useful for later work on domestication potential, conservation and sustainable harvesting. The local name in Libya is “Murrare” (Edama Libya Nature Platform, n.d.).

3.6 rbcL amplification, sequencing and GenBank deposition

PCR of the pooled leaf DNA produced a single amplicon of the expected size (~600 bp), present in lanes P and S and absent from the negative control (lane N) (Fig. 2). A 570-bp partial coding sequence of the plastid *rbcL* gene (sample ID LAZA2) was deposited in GenBank under accession PZ426799. Its conceptual translation (YEV94270.1) is a 190-residue partial *rbcL* protein without internal stop codons, and the record carries the source data of the sampled plants (leaf tissue; Libya: El-Bayda, Omar Al-Mukhtar University campus; collected April 2026). BLAST analysis assigned the sequence unambiguously to *Leontodon*.

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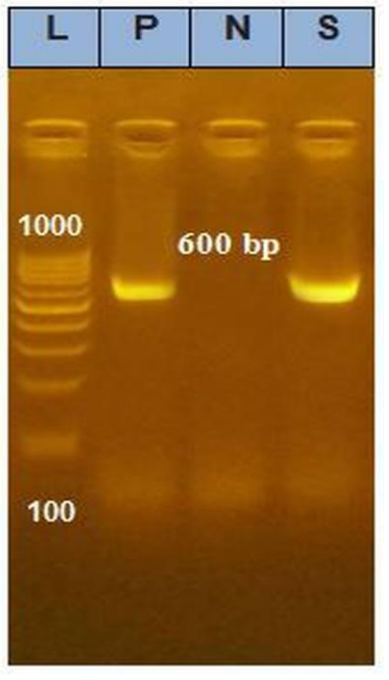


Fig. 2. Agarose-gel (1.5%) electrophoresis of the *rbcL* PCR product amplified from the pooled leaf DNA of *Leontodon tuberosus*. L, ladder (100-bp DNA marker); P, positive control (verifies that the reaction worked); N, negative control (verifies the absence of contamination in the reagents); S, test sample (pooled leaf DNA; sample ID LAZA2). The ~600 bp amplicon is indicated.

3.7 Sequence identity and discriminatory power of rbcL

The LAZA2 sequence was identical to the *L. tuberosus* reference LC911140 along the entire 570-bp alignment (Fig. 3; 100.0% identity, Fig. 4). The closest other taxon, *L. hispidus* (OR990525), differed at a single position (a T; Fig. 3; 99.8% identity, 0.2% divergence). All other taxa were clearly more divergent: identity ranged from 97.9% (*Sonchus asper*) to 98.7% (*Avellara fistulosa*) among the eight other Cichorieae genera and from 97.9% to 98.3% among taxa of other Asteraceae tribes (Fig. 4; Table 6). The divergence between the two *Leontodon* species (0.2%) was thus several-fold lower than the lowest divergence between LAZA2 and

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any other genus (1.3%), and *Leontodon* was resolved separately from *Taraxacum*, *Sonchus*, *Lactuca* and *Cichorium*.



Fig. 3. Nucleotide alignment of the 570-bp *rbcL* region of LAZA2 (PZ426799) and selected reference sequences. Dots indicate identity with the first row (LC911140); letters indicate substitutions. LAZA2 is identical to LC911140, and *L. hispidus* (OR990525) differs at a single position.

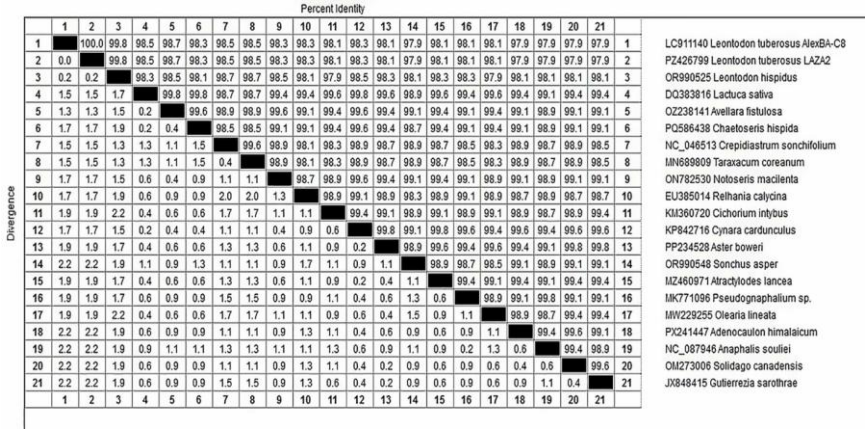


Fig. 4. Pairwise *rbcL* sequence identity (upper triangle, %) and divergence (lower triangle, %) between LAZA2 (PZ426799), the *L. tuberosus* reference (LC911140) and 19 other accessions, calculated in Lasergene DNASTAR (21 sequences in total).

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Table 6. Pairwise *rbcL* identity and divergence between sample LAZA2 (PZ426799) and reference sequences (Lasergene DNASTAR).

Reference taxon	Accession	Identity (%)	Divergence (%)
Leontodon tuberosus (AlexBA-C8)	LC911140	100.0	0.0
Leontodon hispidus	OR990525	99.8	0.2
Avellara fistulosa	OZ238141	98.7	1.3
Lactuca sativa	DQ383816	98.5	1.5
Crepidiastrum sonchifolium	NC_046513	98.5	1.5
Taraxacum coreanum	MN689809	98.5	1.5
Chaetoseris hispida	PQ586438	98.3	1.7
Notoseris macilenta	ON782530	98.3	1.7
Relhania calycina	EU385014	98.3	1.7
Cynara cardunculus	KP842716	98.3	1.7
Cichorium intybus	KM360720	98.1	1.9
Aster boweri	PP234528	98.1	1.9
Atractylodes lancea	MZ460971	98.1	1.9
Pseudognaphalium sp.	MK771096	98.1	1.9
Olearia lineata	MW229255	98.1	1.9
Sonchus asper	OR990548	97.9	2.2
Adenocaulon himalaicum	PX241447	97.9	2.2
Anaphalis souliei	NC_087946	97.9	2.2
Solidago canadensis	OM273006	97.9	2.2
Gutierrezia sarothrae	JX848415	97.9	2.2

Identity and divergence values are given as calculated by Lasergene DNASTAR; the two measures are computed separately by the software and are therefore not necessarily complementary.

Identity with an authenticated reference is consistent with the identification of the Libyan population as *L. tuberosus* and, together with the diagnostic morphology (Fig. 1), with attributing the trait variation reported above to this species. Because the two sequences are identical, their grouping in the tree (Fig. 5) confirms sequence identity and is not independent phylogenetic evidence, and its weight depends on the reliability of the reference record. Because *L. hispidus* differs at a single position (Fig. 3), *rbcL* is not decisive at

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species level, which is consistent with its slow evolution and limited variation among close relatives (Hollingsworth, 2011; Li *et al.*, 2011); species-level identification in this genus is reliable only when a verified *L. tuberosus* reference is present in the comparison set, as it was here. Against a library lacking such a reference, a sequence of this species would be assigned correctly to the genus but could easily be attributed to *L. hispidus*, which illustrates why barcode accuracy is coupled to database completeness (Kress, 2017).

At the generic level the picture is clearer. The gap between intrageneric and intergeneric distances is what makes a locus useful for assigning an unknown specimen to a genus, in line with the expectation that plastid coding regions are informative mainly at higher taxonomic levels in Asteraceae (Panero and Funk, 2008). More variable regions such as *matK*, *ycf1* or nuclear ITS can add resolution among close species (Li *et al.*, 2011; Dong *et al.*, 2015).

3.8 Phylogenetic placement and taxonomic context

The Neighbor-Joining tree of 41 sequences (Fig. 5) placed LAZA2 within *Leontodon*, clustered directly with the *L. tuberosus* reference LC911140 and separate from the two *L. hispidus* accessions (OR990525 and OZ251152); the node uniting the *Leontodon* sequences received 96% bootstrap support. *Leontodon* was resolved separately from the other sampled Cichorieae genera and from genera of other Asteraceae tribes. Branch lengths were very short (scale bar 0.002 substitutions per site), consistent with the strong conservation of *rbcL*, and several deeper nodes received low to moderate support (< 70%) and are not interpreted; the tree was used only for identity confirmation.

The recovery of a *Leontodon* clade distinct from other Cichorieae genera is compatible with the molecular framework for the group, which has shown *Leontodon* in its traditional sense to be non-monophyletic (Samuel *et al.*, 2006). Our taxon sampling is deliberately narrow and not designed to test generic limits, so the tree should not be used to argue for or against any circumscription of the genus; it shows only that the Libyan sequence belongs to the same lineage as the authenticated *L. tuberosus* reference.

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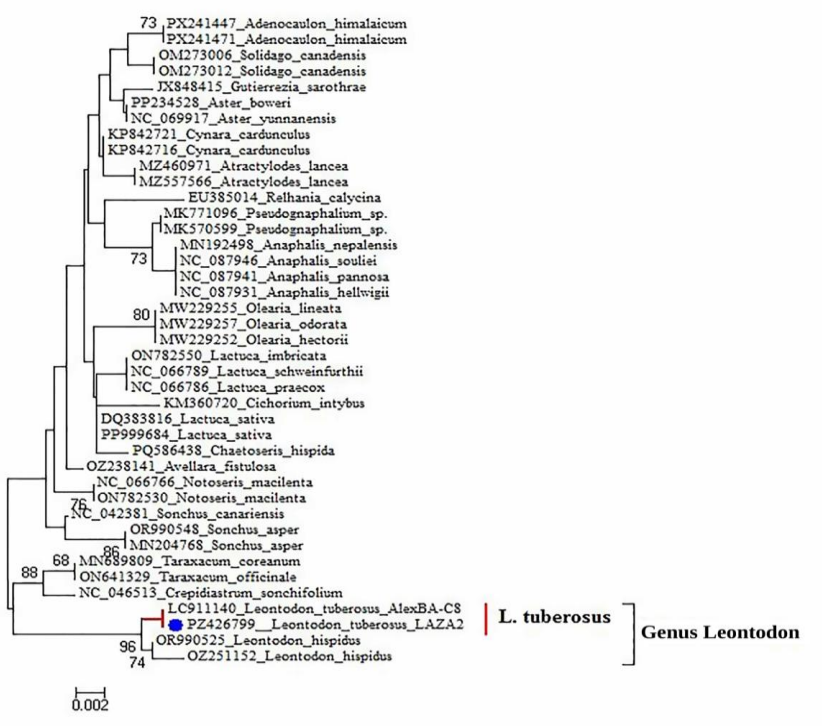


Fig. 5. Neighbor-Joining phylogenetic tree of the plastid *rbcL* gene (41 sequences; Maximum Composite Likelihood model; 1000 bootstrap replicates; bootstrap values shown at the nodes) showing clustering of sample LAZA2 (PZ426799, blue circle) with *Leontodon tuberosus* (LC911140), separate from *L. hispidus* and from other genera of the tribe Cichorieae and the family Asteraceae. Scale bar: substitutions per site.

To our knowledge, accession PZ426799 is the first GenBank record of *L. tuberosus* from Libya, and it adds an independently generated, publicly accessible *rbcL* record from the Al-Jabal Al-Akhdar region to the public reference set. Such records increase the number of reference points against which future specimens can be tested and support the authentication of plant material, a recognised application of DNA barcoding (Raclariu *et al.*, 2018; Kress, 2017).

3.9 Limitations and future work

The study covers 40 plants from a single locality in one season (April 2026); plant positions, ages, and soil and microclimatic

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variables were not recorded, so the results describe associations within this population and cannot be attributed to specific causes or generalised. The published ranges used for comparison come from sources with unstated sample sizes and protocols, and the root-weight model is exploratory. Molecularly, one consensus sequence from pooled leaf DNA represents the population, so intraspecific variation was not assessed; a single maternally inherited plastid locus and one authenticated *L. tuberosus* reference limit species-level resolution, especially where hybridisation has been reported (Chase *et al.*, 2005); the two herbarium vouchers were not sequenced individually, so species identity rests on morphology (Fig. 1); and the search for earlier Libyan records covered GenBank only, so the first-record claim is restricted to it. Future work should sequence individual plants from several populations with herbarium vouchers, add *matK*, *trnH-psbA* and ITS, and broaden taxon sampling across *Leontodon* and allied genera.

4. Conclusions

The quantitative morphology and the molecular analysis of a population of *Leontodon tuberosus* from Al-Jabal Al-Akhdar lead to the following conclusions. (i) The sampled plants displayed the diagnostic tuberous roots, runcinate-pinnatifid leaves, yellow ligulate capitula and plumose-pappus achenes of the species (Fig. 1). (ii) The 40 plants, collected haphazardly from a single locality, provided a quantitative morphological baseline: weight traits (fresh and dry weights of shoots, roots and whole plants) and organ counts varied most (CV 31.0–47.4%), linear dimensions less (CV 19.8–24.6%, except flowering-stalk length, 32.7%) and capitulum diameter least (mean ± SE, 3.62 ± 0.04 cm; CV 7.7%). Leaf count and root traits were positively correlated with organ weights, whereas flower count was not significantly correlated with the other traits and was not predicted by leaf count, leaf length and root length. (iii) A 570-bp *rbcL* sequence (LAZA2, PZ426799) was identical to an authenticated reference sequence, clustered with it with 96% bootstrap support at the *Leontodon* node, differed from *L. hispidus* at one nucleotide and from all other sampled genera by 1.3–

2.2%. The *rbcL* barcode therefore confirms the generic placement of the population and is consistent with the *L. tuberosus* reference, but its resolution relative to the closest congener rests on a single nucleotide difference, so species identity should rely on morphology and, where finer discrimination is needed, on additional loci. The deposited sequence is, to our knowledge, the first GenBank record of *L. tuberosus* from Libya and provides a publicly accessible molecular reference for a Libyan population of the species.

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