

Article

Comparative Taxonomic Study of *Launaea* Cass. (Asteraceae, Cichorioideae) in Egypt

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Abstract: *Launaea* is a polymorphic genus with inter- and intraspecific inconsistencies. The study aimed to revise the classification and identification of *Launaea* species in Egypt. The study also aimed to assess the inter- and intraspecific variation among taxa using macro- and micromorphological characters. Numerical analysis was carried out on 156 specimens, which were collected from 63 sites in Egypt. The results revealed that *L. arabica*, *L. capitata*, *L. intybacea* and *L. spinosa* were significantly (p -value $< 2.2 \times 10^{-16}$ ***) discriminated from the other species at a high dissimilarity level. *Launaea acanthodes* was correctly identified as *Lactuca orientalis*. The study supported the inclusion of *L. intybacea*, *L. massauensis* and *L. nudicaulis* within section *Microrhynchus*, the separation of *L. angustifolia* and *L. arabica* in two distinct species. Two biotypes of *L. nudicaulis* were morphologically differentiated. *Launaea fragilis* subsp. *fragilis* showed considerable plasticity and variability in various characters; therefore, *L. tenuiloba* was considered to be merely an ecotype of *L. fragilis*. *Launaea mucronata* comprises two subspecies; *cassiniana* and *mucronata*, differentiated by their pappus type, conspicuous secondary ribs of the inner achenes, and the number of spines on the polar area of pollen grains.



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Keywords: achenes; asteraceae; *Launaea*; morphology; pollen grains; SEM

1. Introduction

Asteraceae Martynov is the largest and most diverse family in the Eudicots, with 13 subfamilies and 45 tribes. The family consists of about 1600–1700 genera and 25,000–33,000 species [1]. Currently, Susanna et al. [2] recognized 16 subfamilies and 50 tribes. According to APG IV [3], the family was classified into Eudicots, Superasterids, Asterids, Campanulids and Asterales. In Egypt, the Asteraceae is represented by 98 genera, and 234 species are classified into two subfamilies; Liguliflorae and Tubiliflorae. The former is exemplified by two tribes: Cichorieae Lam. and DC, and Scorzonerinae. The Cichorieae contains six subtribes; Crepidinae Cass. ex Dumort., Hypochaeridinae Less., Hyoseridinae Less., Lactucinae (Cass.) Cass. ex Dumort., Rhagadiolinae Benth. and Hook.f. and Scolyminae Less. *Lactuca* L., *Launaea* Cass., *Reichardia* Roth and *Sonchus* L. are grouped in the subtribe Lactucinae [4,5].

Launaea is a polymorphic genus with inter- and intraspecific inconsistencies, especially within section *Zollikoferia* DC. It comprises about 54 species and 10 subspecies and is classified into eight sections. The genus is distributed into different geographical regions, particularly Saharo-Arabian, Irano-Tarmanian and Sudanian S Mediterranean, Africa and SW Asia [6,7]. Most species have economic, ecological, and ethnobotanical importance such as anti-inflammatory, antioxidant, anticancer, children fever, soporific, lactagogue, diuretic, insecticidal, antibacterial and antiparasitic [8–12]. The genus displays considerable diversity in the Mediterranean and Nile regions of Egypt. According to the Egyptian flora, Boulos [5] has recorded ten taxa (9 species and 3 subspecies) and classified them into four sections: *Acanthosonchus* Shultz Bip., *Launaea* Cass., *Microrhynchus* Less. and *Zollikoferia* [5].

The genus was subjected to various taxonomic demarcations and allocations of its species in Egypt, which have been summarized in the Supplementary Materials (Table S1).

The current study aimed to review the classification and identification of *Launaea* species based on an extensive array of macro- and micromorphological characters. The study also aimed to assess the inter- and intraspecific variation among taxa.

2. Materials and Methods

2.1. Collection of Specimens

The study was constructed on 156 fresh and herbarium specimens collected from 63 sites to cover the most dominant habitats within the range of the genus distribution in Egypt (Table S2 and Figure S1). The freshly collected samples were deposited as voucher specimens at Alexandria University Herbarium (ALEX) and identified according to Kilian [7] and Boulos [5]. The herbarium specimens were loaned from Assiut University (ASTU), the Cairo Agricultural Research Center (CAIM), Cairo University (CAI) and the National Research Centre (CAIRC). Herbarium acronyms follow The Index Herbariorum [13]. The studied specimens represent 10 taxa of *Launaea* (*L. angustifolia* subsp. *angustifolia* (Desf.) Kuntze, *L. angustifolia* subsp. *arabica* (Boiss.) N. Kilian, *L. capitata* (Spreng.) Dandy, *L. fragilis* subsp. *fragilis* (Asso) Pau, *L. intybacea* (Jacq.) Beauverd, *L. massauensis* (Fres.) Chiov., *L. mucronata* subsp. *cassiniana* (Jaub. and Spach) N. Kilian, *L. mucronata* subsp. *mucronata* (Forssk.) Muschl., *L. nudicaulis* (L.) Hook.f. and *L. spinosa* Sch.Bip.). *Reichardia* and *Launaea* are the most closely related genera within subtribe Hyoseridinae [14]. As a result, the widely distributed *R. tingitana* (L.) Pau was used as an outgroup in the cluster analysis. Photographs of the whole plant materials were illustrated in the Supplementary Materials (Figures S2–S13).

2.2. Macromorphological Characters and Numerical Analysis

For the vegetative and floral attributes, three replicas were measured for each specimen. Specimens were examined using Olympus stereomicroscope and measured using ImageJ (1.51J8) [15]. All leaf characters were studied for the rosette leaves, except the leaf attachment and the auricle margin were studied in the cauline leaves. For the achene macromorphology, measurements were carried out on nine replicas for each specimen. The studied achenes were selected from three different positions in the fruiting capitulum, marginal, submarginal and inner, to investigate the achenes variation. The submarginal achenes represented a transition state between the marginal and the inner ones; therefore, their measurements were omitted. The achenes measurements were conducted using Olympus stereomicroscope supported with 1 cm ocular micrometer. The terminology of macromorphological characters follows De Vogel [16] Kilian [7], Simpson [17] and Roque et al. [18].

One hundred and fifty-six specimens were subjected to numerical analysis by using 82 macromorphological characters; 16 vegetative, 37 floral and 29 achene characters. The complete list of the assessed quantitative and qualitative macromorphological characters and their states is presented in the Supplementary Materials (Table S3). The agglomerative cluster analysis was performed through the PAST program for mixed data set [19]. The dendrogram was constructed by the UPGMA method of sorting based on the Manhattan dissimilarity coefficient.

2.3. Micromorphological Characters (SEM) and Numerical Analysis

The achene and pollen micromorphological characters were investigated for 17 samples obtained from 17 hypothetical groups. These individuals were selected based on the cluster analysis resulting from the study of 82 macromorphological characters performed to assess the inter- and intraspecific variation among taxa. Marginal and inner achenes were preliminarily examined using a stereomicroscope to ensure that they were of normal size, shape and maturity. The achenes were mounted directly on stubs using double-sided adhesive tape and then coated with gold, up to a thickness of 400 Å in a sputter-coating

unit (JFC-1100 E). The samples were examined and photographed with a JEOL JSM-5300 scanning electron microscope. A total of 11 micromorphological attributes were assessed. The terminology follows Barthlott [20], Simpson [17], Zhang et al. [21] and Hussein and Eldemerdash [22].

The pollen grains were mounted directly on a glass cover and coated with gold up to a thickness of 400 Å in a sputter-coating unit (JFC-1100 E). An average of ten pollen grains were examined and photographed for each specimen with a JEOL JSM-5300 scanning electron microscope. The number of spines on the polar area (excluding ridges), the ratio of polar area to pollen area and the apocolpium index were investigated. The latter was calculated according to the following equation: Apocolpium index = (apocolpium diameter/equatorial diameter) × 100. Fifteen morphological characters were evaluated according to Punt et al. [23] terminology. The complete list of the assessed quantitative and qualitative micromorphological characters and their states were presented in the Supplementary Materials (Table S4).

The cluster analysis was achieved based on 26 micromorphological characters. The similarity among specimens was determined through the Hamming dissimilarity coefficient, and the dendrogram was constructed by the UPGMA method of sorting by using the PAST program of Hammer et al. [19]. Also, a Principal Coordinate Analysis (PCoA) was conducted to visualize similarities and dissimilarities among the 17 groups. The PCoA was performed by using the R- software (Vienna, Austria), with the required packages installed [24].

3. Results

3.1. Numerical Analysis of Macromorphological Characters

Eighty-two macromorphological characters were studied for 156 specimens; 18 vegetative and 37 floral traits (Figure 1), and 29 achenes macromorphological traits (Figures 2 and 3). The performed numerical analysis was illustrated in Figure 4.

Reichardia tingitana was segregated at the highest dissimilarity level (1.420), whereas the taxa of *Launaea* were divided into four groups representing the four corresponding sections: *Zollikoferia* at 1.253, *Acanthosonchus* at 1.134, and both *Launaea* and *Microrhynchus* at 1.013. A total of 17 hypothetical groups were proposed: *R. tingitana*, *Acanthosonchus* (*L. spinosa*), *Launaea* (*L. capitata*), four groups in *Microrhynchus* (*L. intybacea*, *L. massauensis* and two forms of *L. nudicaulis*) and ten groups within *Zollikoferia* (*L. angustifolia* subsp. *angustifolia*, *L. angustifolia* subsp. *arabica*, *L. mucronata* subsp. *mucronata*, *L. mucronata* subsp. *cassiniana* and six forms of *L. fragilis* subsp. *fragilis*). The morphological distinction between these groups is summarized in the Supplementary Materials (Table S5).

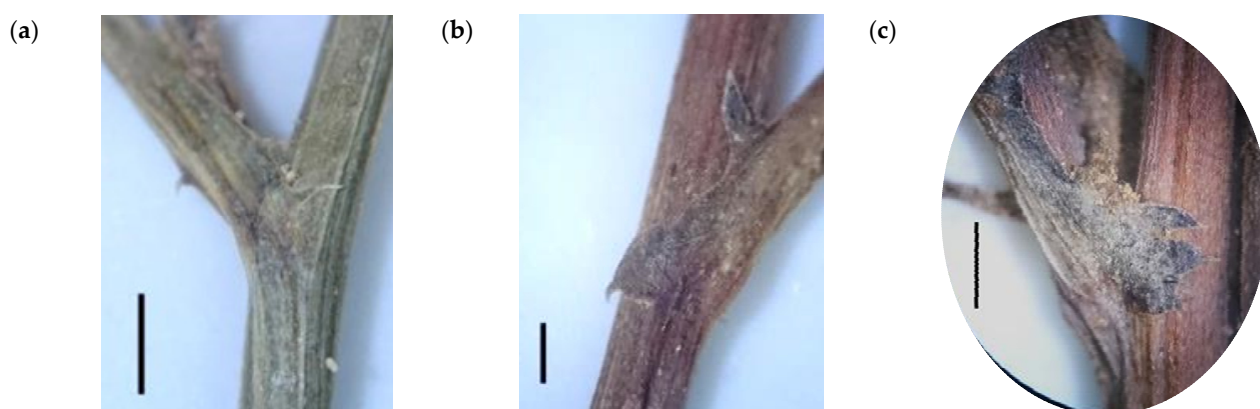


Figure 1. Cont.

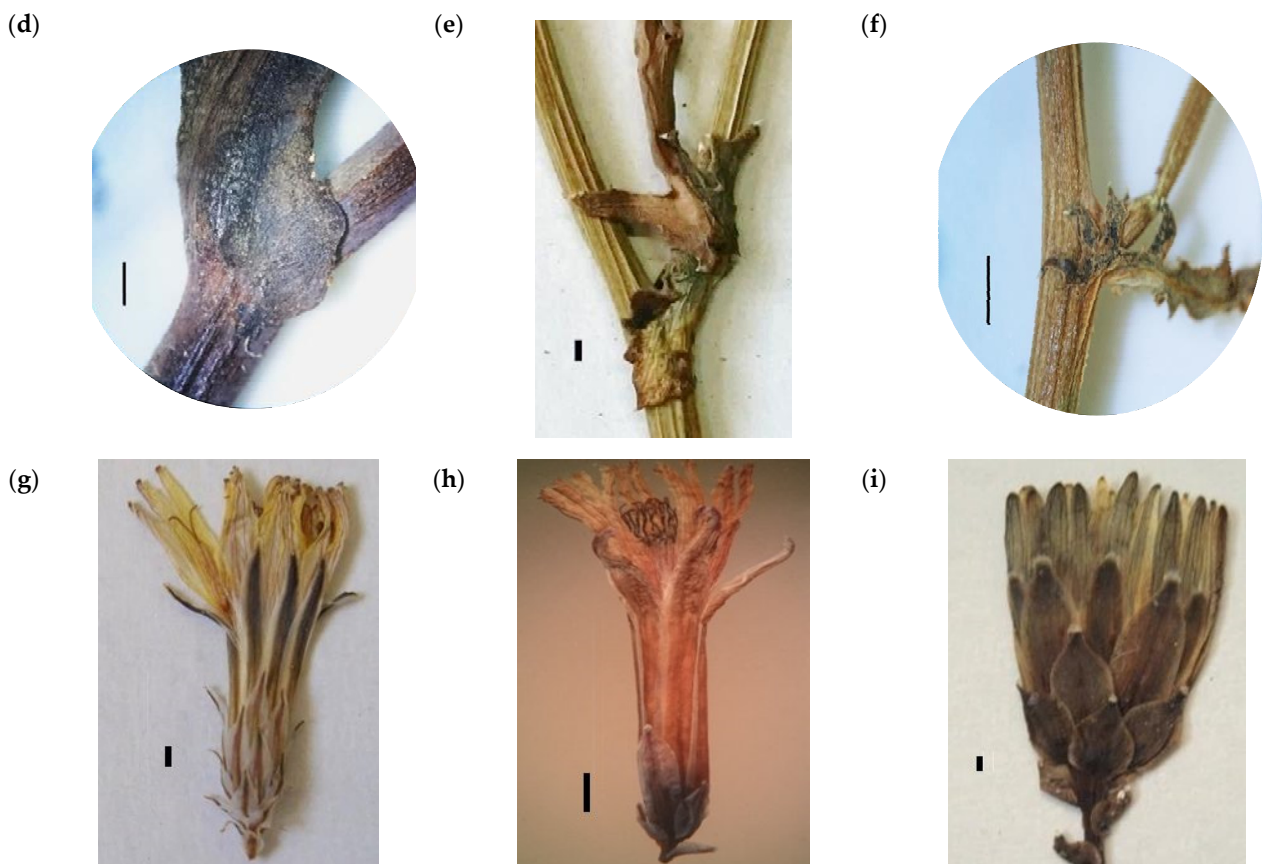


Figure 1. Stereomicrographs of *Launaea* leaves and capitula. (a–f) Leaf attachment and auricle margin; (a) Non-auriculated leaf in *L. nudicaulis*; (b,c) auriculated with semi-amplexicaul base in *L. fragilis* subsp. *fragilis*; (b) entire margin or (c) dentate margin; (d–f) auriculated with amplexicaul base; (d) entire margin in *L. fragilis* subsp. *fragilis* or (e) dentate margin in *L. fragilis* subsp. *fragilis* or (f) dissected margin in *L. mucronata* subsp. *cassiniana*. (g–i) Capitulum shape, involucre scale graduality and ligule color; (g) ellipsoid, non-gradual and yellow ligule; (h) lanceoloid, non-gradual and yellow ligule; (i) obovoid, gradual and yellow ligule with green abaxial surface. Scale bar = 1 mm.

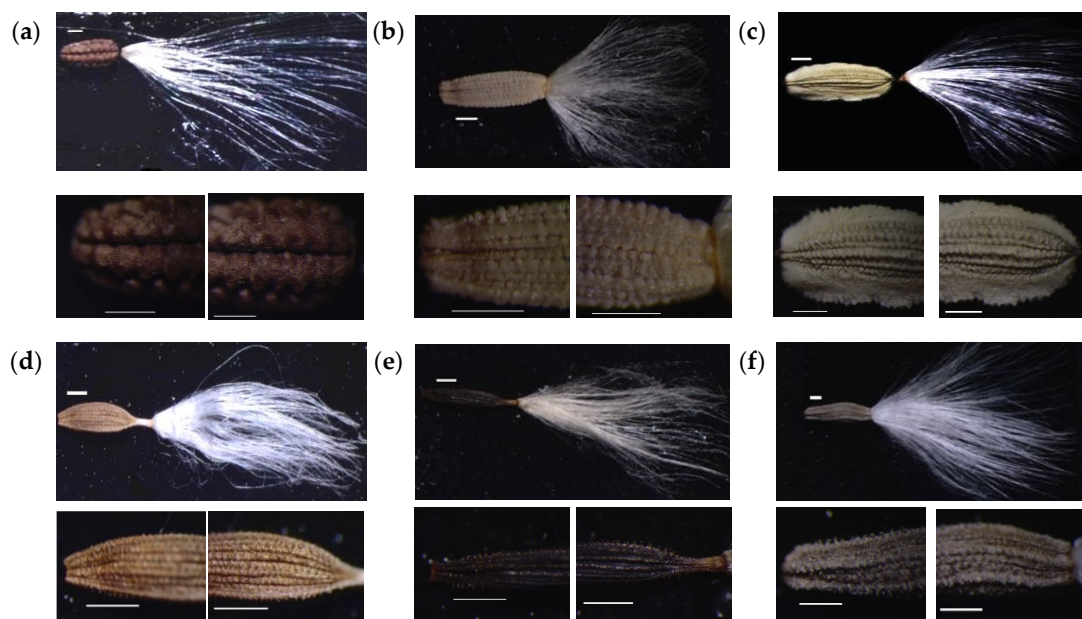


Figure 2. Cont.

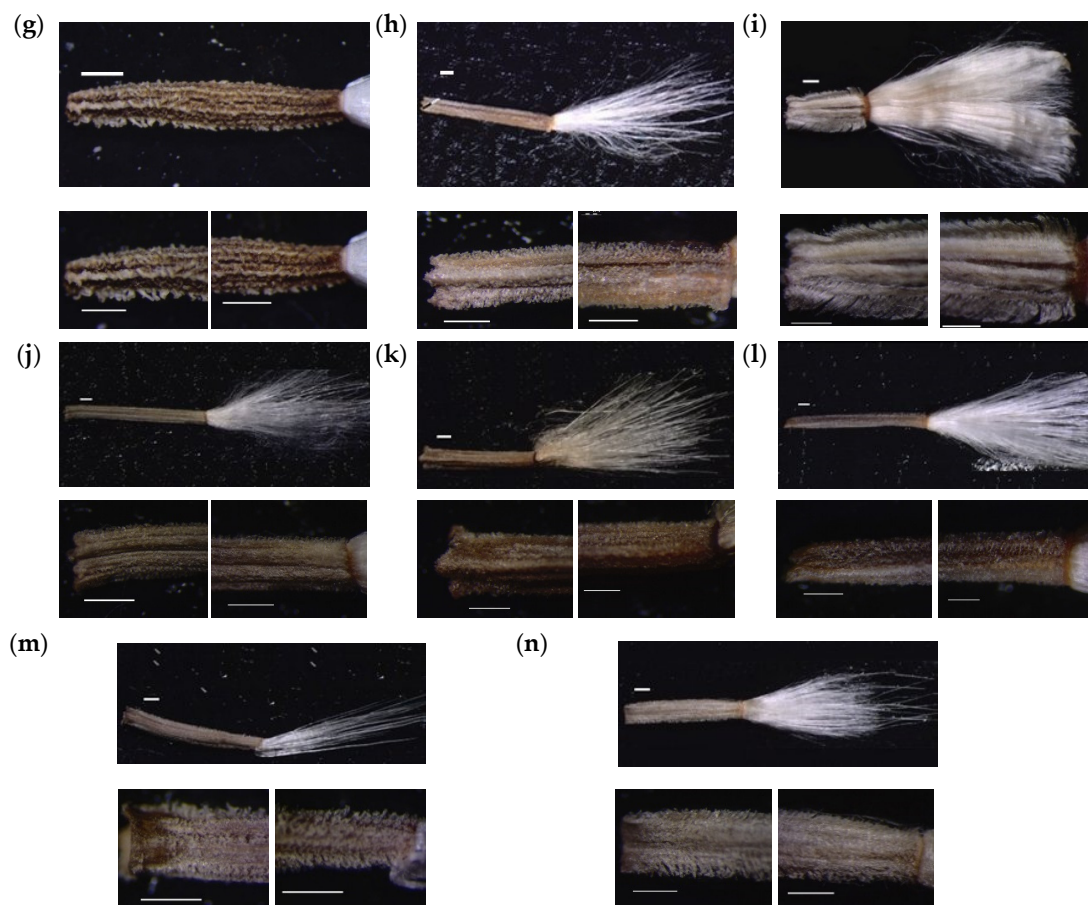


Figure 2. Stereomicrographs of marginal achenes shape (upper), base (lower left) and apex (lower right). (a) *Reichardia tingitana*, obcolumnar, truncate base and apex. (b–n) *Launaea*: (b) *L. spinosa*, subcuneate, truncate base and apex; (c) *L. capitata*, winged-cylindrical, truncate base and apex; (d) *L. intybacea* and (e) *L. massauensis*, beaked-subfusiform, truncate base, rostrate apex; (f) *L. nudicaulis*, subcuneate, truncate base and apex; (g) *L. nudicaulis* (specimen 33), subfusiform, truncate base and apex; (h) *L. angustifolia* subsp. *angustifolia*, cylindrical, horned base, truncate apex; (i) *L. angustifolia* subsp. *arabica*, cylindrical, horned base, truncate apex; (j–l) *L. fragilis* subsp. *fragilis*: (j) cylindrical (specimens 129) or (k) columnar (specimens 72) or (l) obcolumnar (specimens 102), horned base, truncate apex; (m,n) *L. mucronata*: (m) subsp. *cassiniana* and (n) subsp. *mucronata*, columnar, truncate base and apex. Scale bar = 0.5 mm.

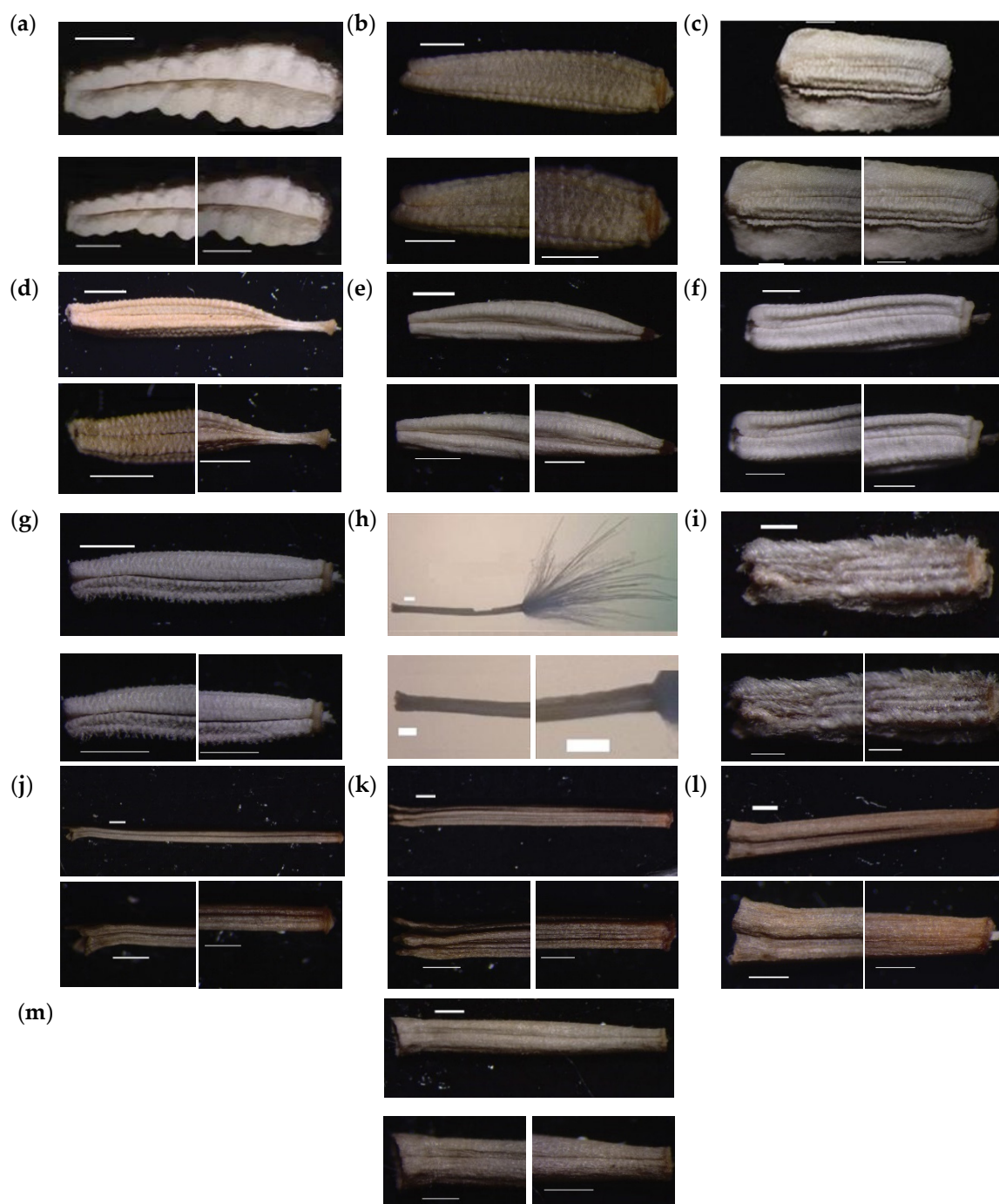


Figure 3. Stereomicrographs of inner achenes shape (upper), truncate base (lower left) and apex (lower right). (a) *Reichardia tingitana*, columnar, truncate base and apex. (b–m) *Launaea*: (b) *L. spinosa*, columnar, truncate base and apex; (c) *L. capitata*, winged-cylindrical, truncate base and apex; (d) *L. intybacea*, beaked-cylindrical, truncate base, rostrate apex; (e) *L. massauensis*, columnar, truncate base, cuspidate apex; (f) *L. nudicaulis*, cylindrical, truncate base and apex; (g) *L. nudicaulis* (specimen 33), columnar, truncate base and apex; (h) *L. angustifolia* subsp. *angustifolia*, cylindrical, horned base, truncate apex; (i) *L. angustifolia* subsp. *arabica*; columnar, horned base, truncate apex; (j,k) *L. fragilis* subsp. *fragilis*: (j) cylindrical (specimens 88) or (k) columnar (specimens 102), horned base, truncate apex; (l,m) *L. mucronata*: (l) subsp. *cassiniana*, (m) subsp. *mucronata*, columnar, truncate base, attenuate apex. Scale bar = 0.5 mm.

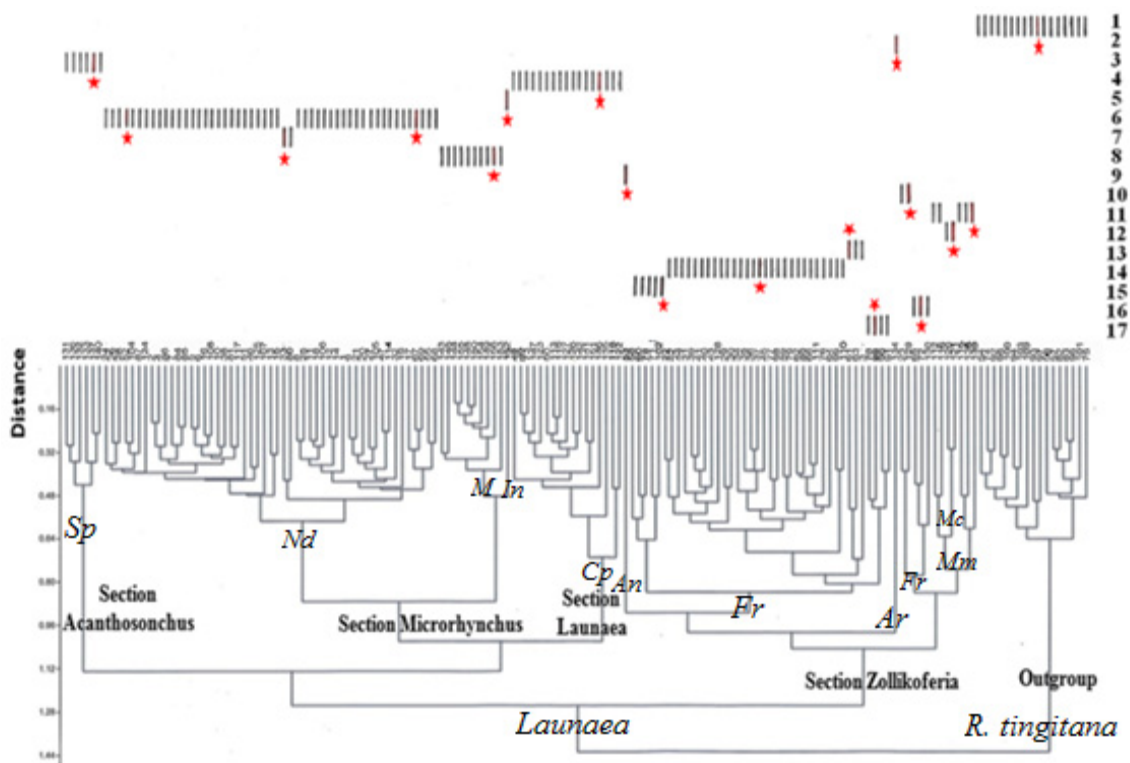


Figure 4. Dendrogram using the UPGMA method of sorting for the 156 specimens of *Launaea* and *Reichardia tingitana*. 1, *R. tingitana*; 2, (*Ar*) *L. angustifolia* subsp. *arabica*; 3, (*Sp*) *L. spinosa*; 4, (*Cp*) *L. capitata*; 5, (*In*) *L. intybacea*; 6–7, (*Nd*) *L. nudicaulis*; 8, (*M*) *L. massauensis*; 9, (*An*) *L. angustifolia* subsp. *angustifolia*; 10, (*Fr*) *L. fragilis* subsp. *fragilis*; 11, (*Mm*) *L. mucronata* subsp. *mucronata*; 12, (*Mc*) *L. mucronata* subsp. *cassiniana*; 13–17, (*Fr*) *L. fragilis* subsp. *fragilis*. Stars indicate the studied specimens for achene and pollen grain micromorphological attributes.

3.2. Numerical Analysis of Micromorphological Characters (SEM)

The agglomerative cluster analysis of 17 samples based on 26 achenes (Figures 5 and 6) and pappus characters (Figure 7), and palynological characters (Figure 8) is illustrated in Figure 9. The highest taxonomic distance of the studied groups was 0.830; at this level, both *R. tingitana* and *L. angustifolia* subsp. *arabica* were discriminated. At 0.655 dissimilarity level, *Launaea* sections were distinguished: *Acanthosonchus*, *Launaea*, *Microrhynchus* and *Zollikoferia*. Both *Acanthosonchus* and *Launaea* were represented by *L. spinosa* and *L. capitata*, respectively. For section *Microrhynchus*, two clusters were segregated: “1” and “2” at 0.420. Cluster “1” aggregated both *L. intybacea* and *L. nudicaulis* (group 6), while cluster “2” grouped *L. massauensis* and *L. nudicaulis* (group 7).

Section *Zollikoferia* was separated into four clusters at 0.50 dissimilarity level. Cluster “3” comprised the two subspecies of *L. mucronata*; *cassiniana* and *mucronata*. While, cluster “4” aggregated groups 13 and 10 of *L. fragilis* subsp. *fragilis*, and the other specimens of the latter subspecies were assembled below cluster “5”. Finally, cluster “6” isolated *L. angustifolia* subsp. *angustifolia*.

The distinction among the seventeen hypothetical groups is performed also by the Principle Coordinate Analysis (PCoA) (Figure 10). The first and second axis explained 31.56% and 28% of the variance, respectively, which emphasize a moderate variation for the data. The taxa representing each section were assembled together. The widest range of variation was noticed in section *Zollikoferia*, especially among the taxa of *L. fragilis* subsp. *fragilis* and between the two subspecies of *L. angustifolia*.

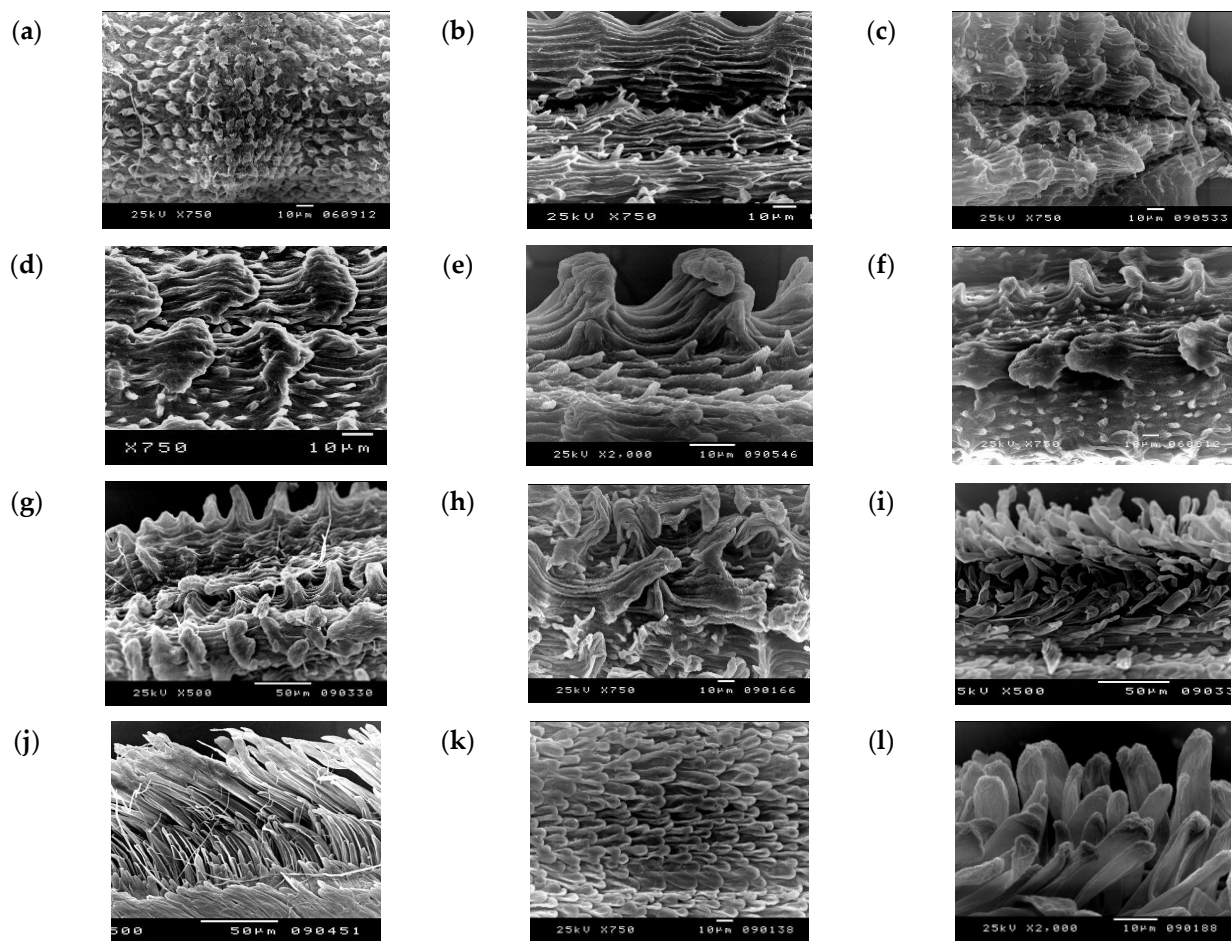


Figure 5. SEM micrographs of marginal achenes. (a) *Reichardia tingitana*, coarse- verrucate transversely rugose surface. (b–l) *Launaea*, wrinkled surface: (b) *L. spinosa*, short squarrose with rounded apex, elongated adjusted epidermal cells with long rounded projections; (c) *L. capitata*, short squarrose with acute apex, elongated adjusted epidermal cells with short rounded projections; (d) *L. intybacea* and (e) *L. massauensis*, long recurved with acute apex, diffused epidermal cells with long acuminate projections; (f, g) *L. nudicaulis* (group 6), long incurved or recurved with acute apex, nearly adjusted elongated epidermal cells with short acute projections; (h) *L. nudicaulis* (group 7), long squarrose with acute apex, nearly adjusted elongated epidermal cells with long acuminate projections. (i–l) *Launaea*, papillose surface: (i) *L. angustifolia* subsp. *angustifolia*, long and linear unconnate papillae; (j) *L. angustifolia* subsp. *arabica*, long and linear connate papillae forming hyaline wing; (k) *L. fragilis* subsp. *fragilis* and (l) *L. mucronata* subsp. *cassiniana*, short and clavate papillae.

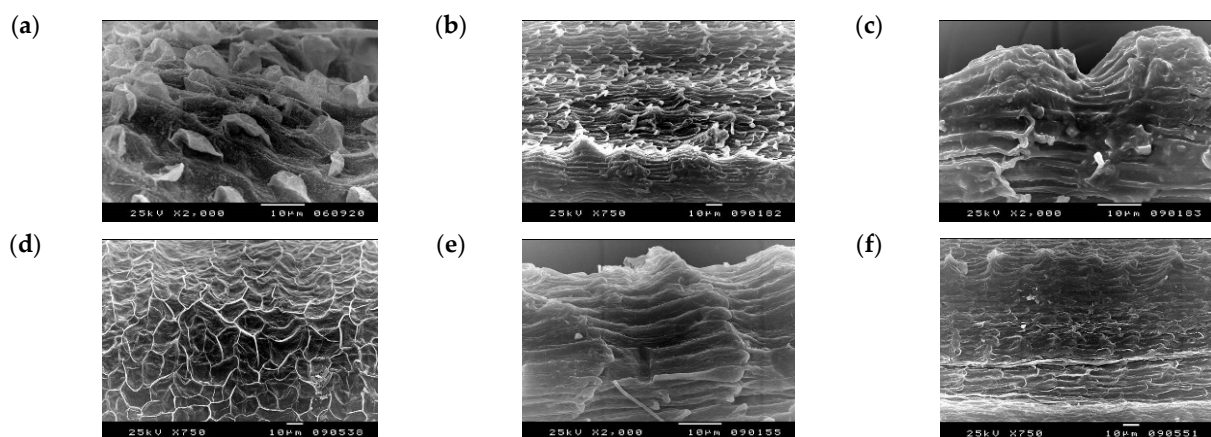


Figure 6. Cont.

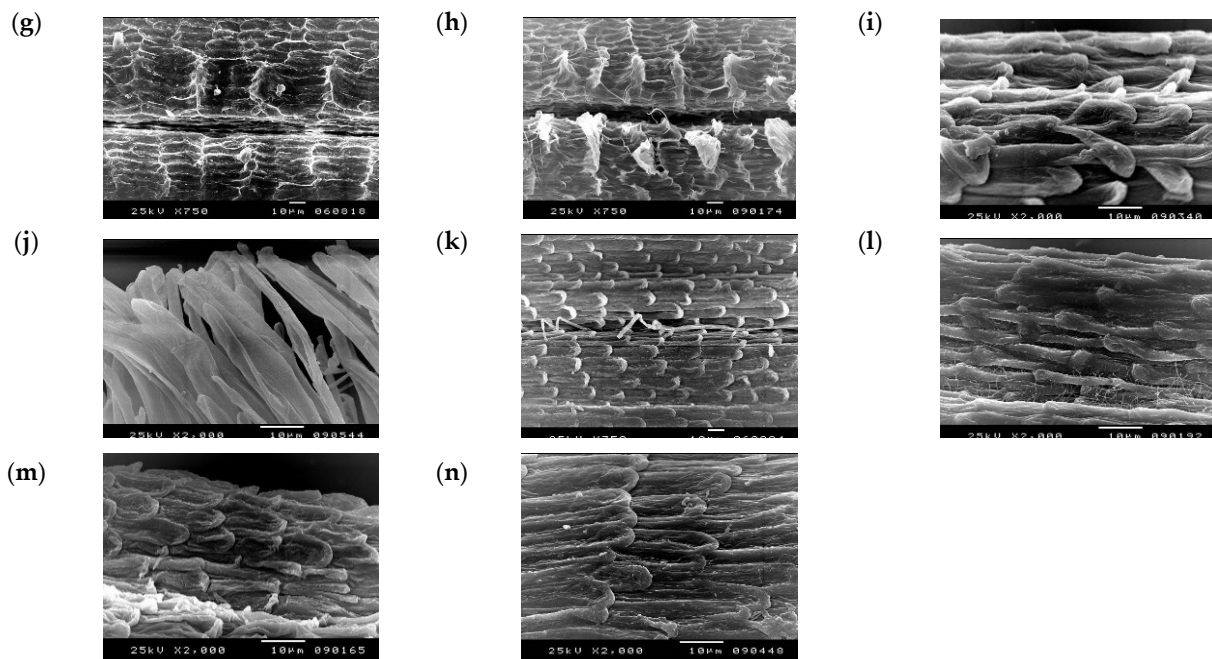


Figure 6. SEM micrographs of inner achenes. Epidermal Cell (EC), End Wall (EW), Anticlinal Wall (AW), Relief of Cell Boundary (RCB), Outer Periclinal Wall (OPW) and Fine Relief of Periclinal Wall (FRPW). (a) *Reichardia tingitana*, isodiametric EC, obscure EW, AW and RCB, sparsely tuberculate OPW, nearly smooth FRPW. (b–n) *Launaea*: (b,c) *L. spinosa*, elongated nearly adjusted EC, long antrorse projection of EW, raised straight AW, channelled RCB, concave OPW, nearly smooth FRPW; (d) *L. capitata*, isodiametric EC, EW without projection (at the achene wing), raised straight to irregular AW, raised RCB, concave OPW, nearly smooth FRPW; (e) *L. intybacea*, elongated adjusted EC, short antrorse projection of EW, raised straight AW, channelled RCB, concave OPW, nearly smooth FRPW; (f) *L. massauensis*, elongated nearly adjusted EC, EW without projection, raised straight AW, raised RCB, concave OPW, nearly smooth FRPW; (g,h) *L. nudicaulis*: EW without projection, raised straight AW, raised RCB, concave OPW, nearly smooth FRPW, (g) group 6, elongated adjusted EC, (h) group 7, elongated nearly adjusted EC; (i) *L. angustifolia* subsp. *angustifolia*, diffused EC, long antrorse projection of EW, raised straight AW, channelled RCB, flat OPW, striate FRPW; (j) *L. angustifolia* subsp. *arabica*, obscure EC, EW, AW and RCB, nearly smooth FRPW, papillose OPW; (k,l) *L. fragilis* subsp. *fragilis*, (k) nearly adjusted EC, long antrorse projection of EW, raised straight AW, channelled RCB, flat OPW, nearly smooth FRPW; (l) diffused EC, short antrorse projection of EW, raised straight AW, raised RCB, concave OPW, striate FRPW; (m,n) *L. mucronata*: elongated adjusted EC, raised straight AW, channelled RCB, flat OPW, striate FRPW, (m) subsp. *cassiniana*, long antrorse projection of EW, (n) subsp. *mucronata*, short antrorse projection of EW.

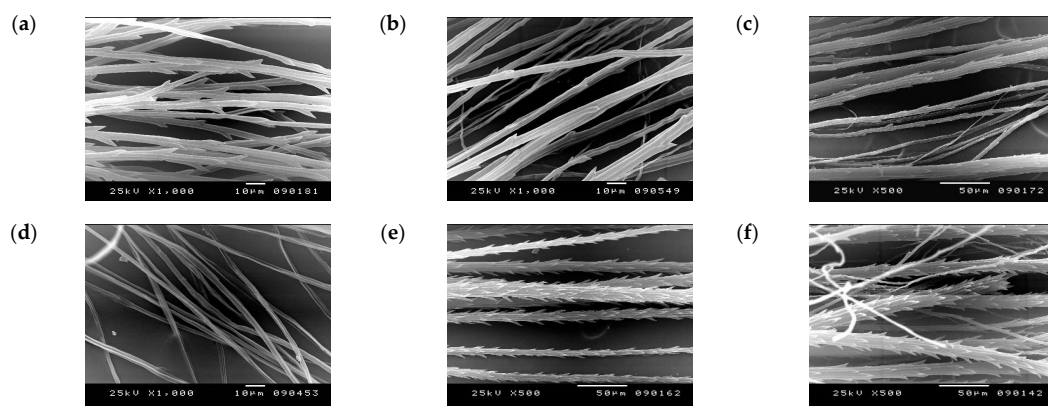


Figure 7. SEM micrographs of pappus type, protuberance density in 150 µm and orientation. (a) subhomomorphic setaceous bristles, sparsely, appressed in *L. spinosa*; (b) dimorphic, sparsely, appressed in *L. massauensis*; (c) dimorphic, moderate, appressed in *L. nudicaulis* (group 7); (d) homomorphic of cottony rays, bristles absent in *L. angustifolia* subsp. *arabica*; (e,f), high, inclined in *L. mucronata*: (e), subhomomorphic setaceous bristles in *L. mucronata* subsp. *cassiniana*, (f), dimorphic in *L. mucronata* subsp. *mucronata*.

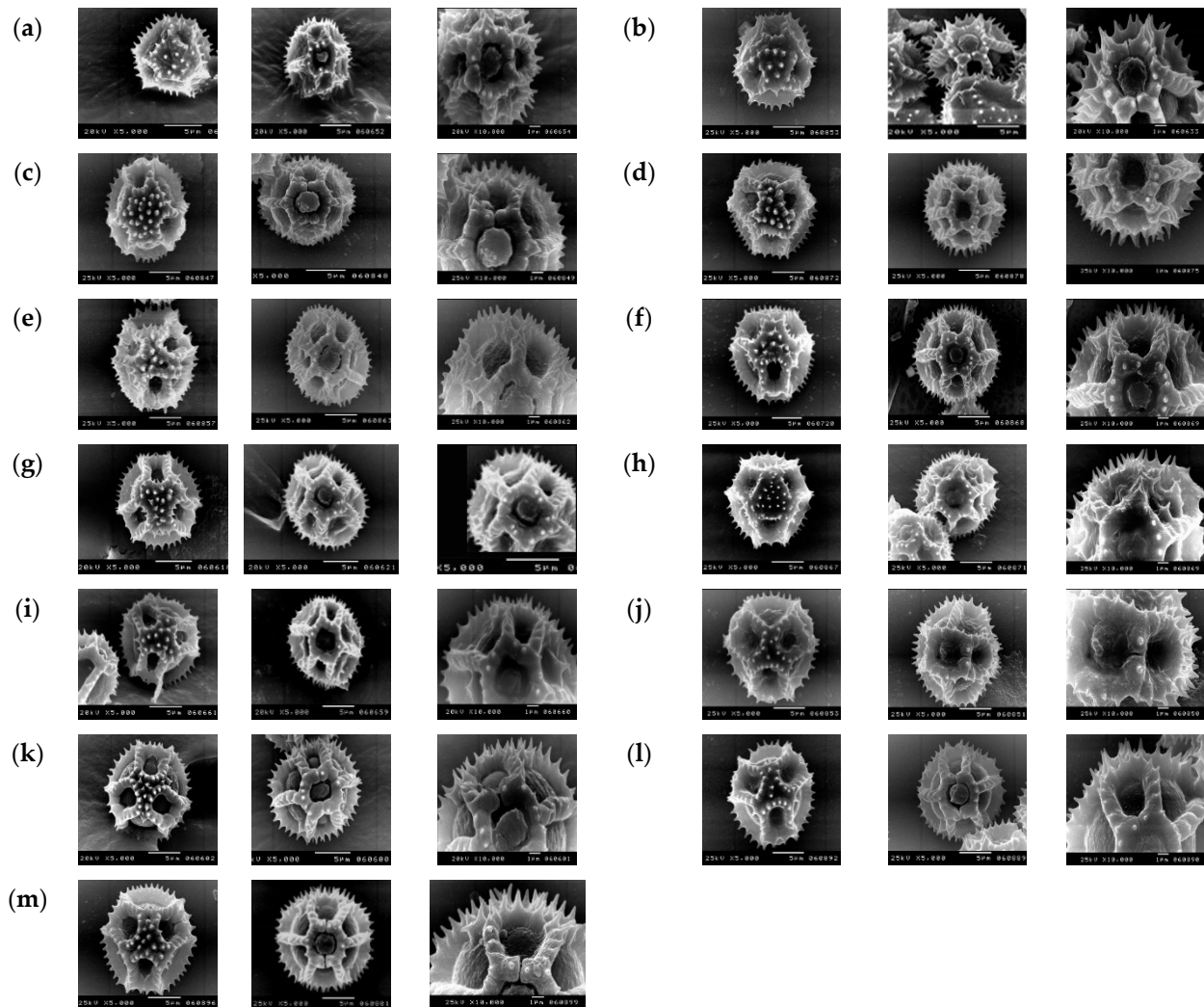


Figure 8. SEM micrographs of pollen grains for polar view (left), equatorial view (middle) and paraparal ridge ornamentation (right) for each taxon. **(a)** *Reichardia tingitana*, spiny-granulate. **(b–m)** *Launaea*: **(b)** *L. spinosa*, spiny; **(c)** *L. capitata*, spiny-perforate; **(d)** *L. intybacea* and **(e)** *L. massauensis*, spiny-perforate; **(f,g)** *L. nudicaulis*: **(f)** group 6, spiny-perforate; **(g)** group 7, spiny; **(h)** *L. angustifolia* subsp. *angustifolia*, spiny-perforate; **(i)** *L. angustifolia* subsp. *arabica*, spiny; **(g–k)** *L. fragilis* subsp. *fragilis*, **(g)**, group 13, spiny-granulate, **(k)**, group 17, spiny-perforate; **(l)** *L. mucronata* subsp. *cassiniana*, spiny; **(m)** *L. mucronata* subsp. *mucronata*, spiny-perforate.

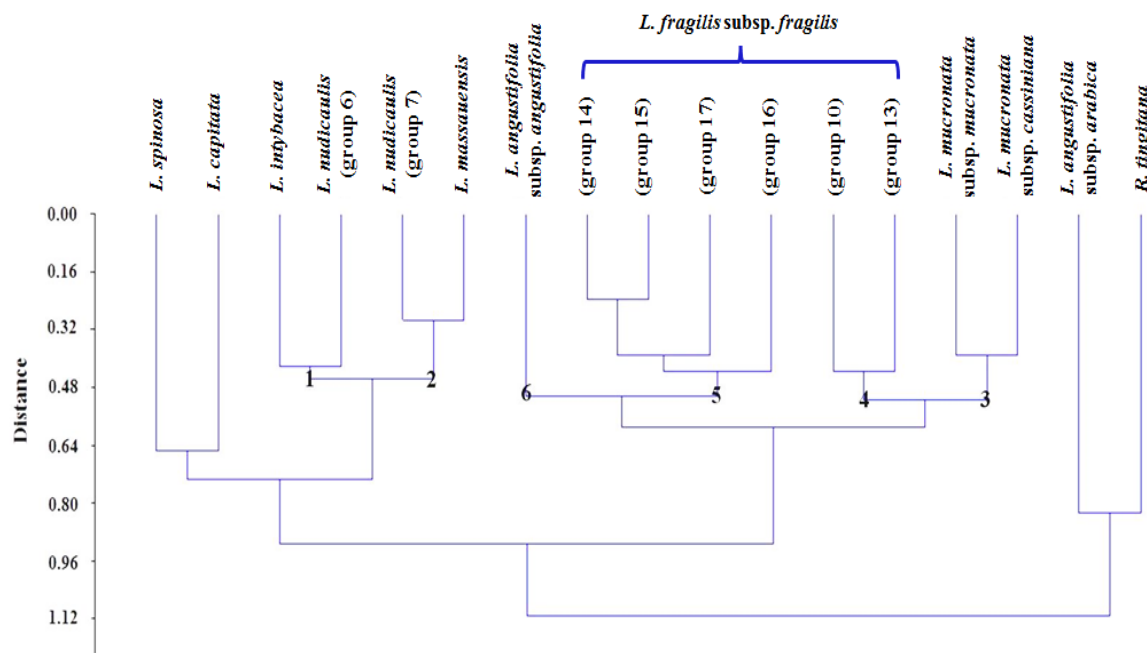


Figure 9. Dendrogram resulted from the UPGMA method of sorting for seventeen samples representing the outgroup *Reichardia tingitana* and 10 taxa of *Launaea* based on the achene and the pollen grain micromorphological attributes.

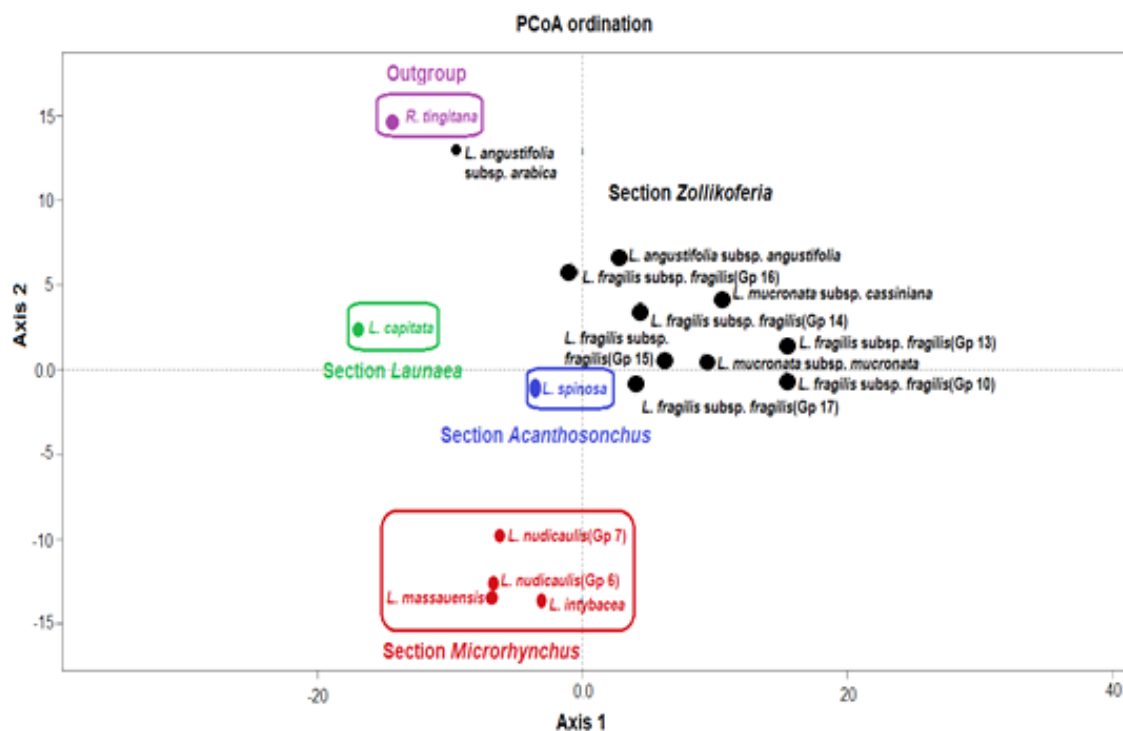


Figure 10. Principal Coordinate Analysis (PCoA) of seventeen samples representing the outgroup *Reichardia tingitana* and 10 taxa of *Launaea* based on the achene and the pollen grain micromorphological attributes.

4. Discussion

The current study is the first comprehensive taxonomic study on *Launaea* in Egypt based on morphological data. *R. tingitana* was used as an outgroup. The outgroup can be defined as a more distantly related group of taxa that serves as a reference group when determining the phenetic relationships of the in-group [25]. According to Kilian

et al. [14], *Reichardia* and *Launaea* are the most closely related genera within Hyoseridinae. The detailed characterization of *Launaea* sections and the species in each one, as well as the taxonomic status for each species, will be summarized in the following part.

4.1. Section *Acanthosonchus*

It is a monospecific section containing *L. spinosa*, although Zareh et al. [26] have added *L. acanthodes* (Boiss.) Kuntze as a new record, based on one herbarium specimen (kept in ASTU herbarium) from Wadi Telah in Sinai (7.10.1983). In the current study, this specimen is correctly identified as *Lactuca orientalis* (Boiss.) Boiss. (Syn. *Scariola orientalis* Boiss.), which is characterized by spinescent shrub and woolly-floccose white stems of green stripes, as well as the sessile heads with up to 5 florets per capitulum and the achene of 8 main ribs on each face with scabridulous pappus [4,5,27]. That is in agreement with Kilian [7], as *Lactuca orientalis* is confusing and similar to *L. acanthodes*.

4.2. Section *Launaea*

Osman [28] and Soliman et al. [29] were accepted by only *L. capitata* in the section. *Launaea procumbens* (Roxb.) Ramayya and Rajagopal was added as a very rare species by Montasir and Hassib [30], Täckholm [4], Kilian [7] and Boulos [5]. The existence of *L. procumbens* in Egypt is based on four specimens collected from Wadi Mbaruth (Qoseir), El-Tor (Sinai), Cairo (Pyramids) and Karnak (Luxor) since 1864, 1880, 1902 and 1926, respectively [5,7]. Currently, this species is represented by three sterile specimens collected in 1956 from Wadi Abar (Gebel Ataqa), Wadi Seyal (North Galalah), and Wadi Qiseib (Suez), which are kept in the CAI Herbarium. Therefore, the current study includes only *L. capitata* in section *Launaea*.

4.3. Section *Microrhynchus*

Launaeaintybacea, *L. massauensis* and *L. nudicaulis* belong to section *Microrhynchus*.

Launaea intybacea was translocated from *Lactuca* to *Launaea* as *Launaea fallax* or *Launaea goraensis* [4,30]. It is a very rare species, and it was not recorded in the Egyptian collection of Kilian [7] and Boulos [5]. The present study includes a unique sample deposited in the CAI Herbarium. The sample was collected from Wadi Laseitit (Halayeb Triangle) by Täckholm in 1962.

For *Launaea massauensis*, the results support involving it with *L. intybacea* and *L. nudicaulis* in the same section. This finding corresponds to Kilian [7] for assorting *L. massauensis* in *Microrhynchus* instead of *Brachyrrhampus* DC, as was classified by Boissier [31].

Launaea nudicaulis is a common polymorphic species in Egypt [4,5,30]. The investigation of the vegetative and floral characters reveals significant variation resulting from many intermediate forms. The most distinguished characters are the leaf size and shape, capitulescence type, floret dimensions, achene size, color and the degree of wrinkles that decrease in the inward direction. The description coincides with the studies of Amin [6], Kilian [7] and Alavi [32], where the achenes are variable in width, color and degree of wrinkles and achieve a gradual decrease towards the head center.

Two biotypes are distinguished morphologically: biotype 1 (group 6) and biotype 2 (group 7). For biotype 1, the capitulescence is either divaricate or divaricate with a cluster. The marginal achenes are subcuneate of $2.05\text{--}3.35 \times 0.30\text{--}0.70$ mm, with long incurved or recurved wrinkles of acute apex, and epidermal cells among wrinkles with short acute projections. The inner achenes are cylindrical of $2.05\text{--}3.40 \times 0.25\text{--}0.60$ mm. They are either compressed or uncompressed with elongated adjusted epidermal cells. The pappus is relatively short, of 5.85 to 9.95 mm, and the bristles reveal four different thicknesses with sparse protuberance density. The size of the pollen grains is $13.19\text{--}13.99 \times 14.28\text{--}14.92$ μm . The equatorial ridge varies from 4.89 to 5.56 μm , and the apocolpium diameter from 4.10 to 4.48 μm . The depth of abporal lacunae ranges from 2.31 to 2.71 μm , while that of paraporal lacuna is 1.97 to 2.50 μm . The paraporal ridge ornamentation is either spiny-perforate or spiny-granulate, and the number of spinules on the polar area ranges from 13 to 17.

For biotype 2, the capitulescence is divaricated. The marginal achenes are subfusiform of $2.40\text{--}3.90 \times 0.30\text{--}0.65$ mm, with long squarrose wrinkles of acute apex, and long acuminate projections of the epidermal cells among wrinkles. The inner achenes are compressed and columnar of $2.15\text{--}3.30 \times 0.30\text{--}0.50$ mm, and their epidermal cells are elongated nearly adjusted. The pappus ranges from 7.15 to 10.30 mm, and its bristles possess five different thicknesses of moderate protuberance density. The size of the pollen grains is $11.74\text{--}12.34 \times 11.52\text{--}13.12$ μm , the equatorial ridge is from 5.46 to 5.69 μm , and the apocolpium diameter is from 3.21 to 4.39 μm . The depth of abporal lacunae varies from 2.29 to 2.40 μm , whereas the depth of paraporal lacuna ranges from 1.97 to 2.04 μm . The paraporal ridge ornamentation is spiny, and the number of spinules on the polar area ranges from 13 to 15. The achene macromorphological characters of biotype 2 are congruent with the description of *L. procumbens*, mentioned by Alavi [32], Kilian [7], Boulos [5] and Qureshi et al. [33], excluding the deciduous homomorphic pappus.

Therefore, the present study suggests two biotypes of *L. nudicaulis*, one of which shares some macromorphological characters with *L. procumbens*, such as the subfusiform marginal achenes, the columnar inner achenes and the dimorphic pappus [34]. Determining the taxonomic rank of these biotypes requires freshly collected specimens for *L. procumbens* and more specialized molecular techniques, such as DNA barcoding, amplified fragment polymorphism (AFLP), or microsatellites.

4.4. Section *Zollikoferia*

According to the Egyptian taxa, the section contained three subgroups: amal-aminiae, angustifolia and mucronata [7].

In the first subgroup, *L. amal-aminiae* N.Kilian was recognized by Amin [35] as a distinct and undescribed species. Kilian [7] identified this species according to its leafless flowering stems, slender capitula, and the different indumentum of the achenes. This description was based on five old specimens collected from Sinai, El-Arish, and Wadi Tundebar in Ras Benass (Red Sea) in 1849, 1855 and 1864, respectively. Besides, two specimens were collected from Suez since 1886 and 1908. Through personal communication with Prof. Amal Amin, she clarified that there were two voucher specimens representing this species, kept at the CAI Herbarium. These two specimens were collected from Suakin el Qadim, and from Halayeb Triangle. Nowadays, the two specimens no longer exist at the CAI Herbarium; also Suakin el Qadim became a part of Sudan. As a result, this species was not involved in the present study. The second subgroup, angustifolia, was represented in Egypt by *L. angustifolia* Muschl. with two subspecies: *angustifolia* and *arabica*. *Launaea angustifolia* subsp. *angustifolia* was first recorded in Egypt by Zareh et al. [26,36], whereas Kilian [7] declared that the species is restricted to a small area in NW Africa, and it had not been found in Egypt. Our study characterizes the subspecies with its papillose marginal achenes that showed long unconnate papillae in the form of two wings, glabrous inner, and achenes dimorphic pappus. An extensive field survey must be done to address the distribution of this species in Egypt.

Following Muschler [37], Montasir and Hassib [30], Täckholm [4] and Boulos [5], the description of *L. angustifolia* in Egypt has been applied to the subspecies *arabica*. Kilian [7] accepted *Launaea arabica* (Boiss.) H.Lindb. at the subspecies level; *L. angustifolia* subsp. *arabica* (Boiss.) N.Kilian. Whereas the current study considered *L. arabica* at the species level, that is confirmed through the numerical analyses using all types of data, separately and collectively. Marginal and inner achenes of *Launaea arabica* display long and linear connate papillae (hyaline papillae), and homomorphic pappus of cottony rays.

The third subgroup mucronata comprises both *L. fragilis* and *L. mucronata*. The two species are closely related with relative uniformity and some infraspecific variability [7]. Both taxa were treated as conspecific and were ranked as subspecies or varieties by various authors [32,38,39].

Launaea fragilis is characterized by their auriculated cauline leaves, semiamplexicaul or amplexicaul base, and auricle of either entire or dentate margin. The apices of the outer and

middle involucre scales may be acute or acuminate. The inner achenes exhibit conspicuous secondary ribs, and horned base. The pappus is dimorphic with less than 100 setaceous bristles of moderate appressed aristate protuberance, while *L. mucronata* is specified with auriculated cauline leaves, amplexicaul base, and dissected auricle margin. The apices of the outer and middle involucre scale are acuminate. The inner achenes show ventricose truncate base with either conspicuous or inconspicuous secondary ribs. The pappus is subhomomorphic of setaceous bristles or dimorphic with less than 100 setaceous bristles. The bristles possess inclined aristate protuberance of high density.

The cluster analysis of the micromorphological characters illustrates the separation of groups 10 and 13 of *L. fragilis* subsp. *fragilis* with *L. mucronata*. These groups share some characters, such as the raised straight anticlinal walls of inner achenes, the channelled relief of cell boundary, and the flat outer periclinal walls with striate fine relief. Also, the ratio of polar area to pollen area is less than 40%, and the length spines is more than 1 μm . Some individuals of *L. fragilis* share characters with *L. mucronata*, such as the auriculated leaves, the large capitula, and the ventricose base of the inner achenes. These forms were initiated due to the hybridization between the two species, as they were sympatric in the Nile Delta and the Mediterranean coast in Egypt. In these regions, two cytotypes of *L. fragilis* were identified, diploid ($2n = 16$) and tetraploid ($2n = 32$) [7,29,40].

Launaea fragilis subsp. *fragilis* has demonstrated considerable plasticity and variability in many attributes related to its leaf, capitulum size, number of florets, achene and pappus. These variations were correlated with the ecological amplitude and the wide geographical distribution of the subspecies. Therefore, several names were published at the specific or the infraspecific rank, validly or invalidly, such as *L. longiloba* (Boiss. and Reut.) Maire, *L. resedifolia* Druce or *L. tenuiloba* Muschl. [4,30,41,42].

In Egypt, *L. resedifolia* was cited as a polymorphic species characterized with linear leaf lobes, the main ribs of the marginal achene are 5 to 6, and a silky pappus with a length up to 12 mm [4,6,32]. González [43] accepted the name *L. fragilis* for *L. resedifolia* and pointed out that the basionym of *L. resedifolia* has belonged to *Scorzonera laciniata* L. *L. longiloba* was ranked as an ecotype for *L. fragilis* and attributed its vigorous plants to the humidity and favorable conditions by Kilian [7]. Täckholm [4] and Feinburn-Dothan [44] classified *L. tenuiloba* at the species level. They have described this species by its filiform leaves with entire or dentate lobes, pappus length that is equal to the achene, and its inland distribution. Zareh et al. [26] allocated *L. tenuiloba* as a subspecies: *L. fragilis* subsp. *tenuiloba* (Boiss.) Zareh and Mohamed comb. et stat. nov., based on its various leaf lobes, and the pappus that is longer than the achene.

Our findings discriminate six intermediate forms correlated to the environmental factors without specific features. Consequently, the current study consents *L. longiloba*, *L. tenuiloba* and the intermediate forms as ecotypes in *L. fragilis* subsp. *fragilis*.

Launaea mucronata Muschl. comprised two subspecies: *cassiniana* and *mucronata* [5,7]. They were ranked at the species level by Montasir and Hassib [30] and Täckholm [4]. However, Alavi [32] accepted this species as a variety of *L. resedifolia*. In light of the studied macro- and micromorphological attributes, the subspecies level is accepted; *L. mucronata* subsp. *cassiniana* and *L. mucronata* subsp. *mucronata*.

Launaea mucronata subsp. *cassiniana* has lanceolate rosette leaves, and the capitulum (5.70–5.90 \times 4.20–5.03 mm) contains about 51 to 56 yellow florets. The compressed inner achenes have inconspicuous secondary ribs, and the epidermal cells have long antrorse projections. The pappus is easily removed and subhomomorphic with the setaceous bristles ranges from 20 to 30 and exhibits three different thicknesses. However, many authors have pointed out the presence of homomorphic pappus rather than the subhomomorphic type [4,5,31,45]. The pollen grain ornamentation on the paraporal ridge is spiny, and the number of spines on the polar area ranges from 3 to 5.

Launaea mucronata subsp. *mucronata* possesses oblong to lanceolate rosette leaves, and the capitulum (9.64–17.15 \times 4.06–10.26 mm) encloses about 35 to 60 yellow florets. The florets have a green abaxial surface. The inner achenes are uncompressed with conspicuous

secondary ribs, and the epidermal cells have short antrorse projections. The pappus is dimorphic and made of up to 40 setaceous bristles with five different thicknesses. Although, Täckholm [4], Amin [6] and Boulos [5] had recorded only 12 to 13 bristles. The pollen grains display a spiny-perforate paraporal ridge, and the number of spines is about 10 to 14 spines on the polar area, the number of spines counted by Osman [28] for the two subspecies agrees with the present study.

The current study proposes a dichotomous indented key for the discrimination and identification of taxa of *Launaea* based on macro- and micromorphological characters.

1. Spinescent branches; early deciduous cauline and rosette leaves, leaf lobes 4; achenes subhomomorphic, inner achene with 5 main ribs *L. spinosa*
1. Spineless branches; persistent rosette leaves, leaf lobes more than 5; achenes heteromorphic, inner achene with 4 main ribs 2
2. Leaf fragile; marginal achene apex rostrate and beaked 3
2. Leaf normal; marginal achene apex truncate and not beaked 4
3. Leaf lobe shape ovate; capitulescence divaricate; capitulum width less than 2 mm; inner achene shape columnar and creamy white with apex cuspidate *L. massauensis*
3. Leaf lobe shape oblong; capitulescence virgate; capitulum width more than 2.5 mm; inner achene shape cylindrical and straw with apex rostrate *L. intybacea*
4. Capitulescence glomerate; ligule whitish-yellow; winged achenes, pappus deciduous *L. capitata*
4. Capitulescence diffused, divaricate, or divaricate with cluster; ligule yellow; unwinged achenes, pappus persistent 5
5. Cauline leaves non-auriculated, rosette leaves runcinate; involucre scales not gradual in length, capitulum shape ellipsoid or lanceoloid; marginal achene shape subcuneate or subfusiform with wrinkled surface, inner achene creamy white *L. nudicaulis*
5. Cauline leaves auriculated, rosette leaves spathulate; involucre scales gradual in length; capitulum shape obovoid; marginal achene cylindrical or columnar or obcolumnar with papillose surface, inner achene brown 6
6. Rosette leaf lobed, leaf lobe shape ovate; peduncle length less than 1 cm; marginal achene length (2.37–2.5 mm), inner achene length (2.56–2.73 mm), marginal and inner achenes papillose with long and linear connate papillae (hyaline papillae), pappus homomorphic of cottony rays *L. arabica*
6. Rosette leaf partite or dissected, leaf lobe shape oblong to linear; peduncle length more than 1.5 cm; marginal achene length (3.20–7.73 mm), inner achene length (3.47–8.40 mm), marginal achenes only papillose with short and clavate or long and linear unconnate papillae, pappus dimorphic or subhomomorphic of setaceous bristles 7
7. Ligule shape linear; marginal achenes with long and linear unconnate papillae; polar area to pollen area ratio >40%, spinules number on polar area from 16 to 20 *L. angustifolia*
7. Ligule shape oblong to linear-lanceolate; marginal achenes with short and clavate papillae; polar area to pollen area ratio <40%, spinules or spines number on polar area from 3 to 14 8
8. Auricle margin entire or dentate; inner achene with conspicuous secondary ribs and 4-horned (toothed) base *L. fragilis* subsp. *fragilis*
8. Auricle margin dissected; inner achene with conspicuous or inconspicuous secondary ribs and ventricose truncate base 9
9. Ligule yellow with green abaxial surface; marginal achene length (2.95–4.0 mm), marginal achene length to width from 5.1 to 7.0, inner achene length (3.40–4.60 mm), inner achene uncompressed with conspicuous secondary ribs, pappus dimorphic; spines number on polar area from 10 to 14 *L. mucronata* subsp. *mucronata*
9. Ligule yellow; marginal achene length (4.10–4.65 mm), marginal achene length to width from 9.5 to 13, inner achene length (5.10–5.60 mm), inner achene compressed

with inconspicuous secondary ribs, pappus subhomomorphic of setaceous bristles; spines number on polar area from 3 to 5 *L. mucronata* subsp. *cassiniana*

5. Conclusions

The combined investigations of the macro- and micromorphological traits are used to revise the classification of ten taxa of *Launaea* and to assess the inter- and intraspecific variation among taxa. The authors suggest the following points: (1) The taxonomic rank of *Launaea angustifolia* subsp. *arabica* must be raised to the species level. It is identified in the present study as *Launaea arabica*. (2) The elimination of *L. acanthodes* from the Egyptian flora, as it is identified correctly as *Lactuca orientalis* (Syn. *Scariola orientalis*). (3) Two biotypes of *L. nudicaulis* are distinguished morphologically. One of them shares some macromorphological characters with *L. procumbens*. *Launaea procumbens* needs further morphological and molecular investigations using freshly collected materials. (4) *Launaea fragilis* subsp. *fragilis* displays considerable plasticity and variability in various characters with intermediate forms, so they are considered ecotypes.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/taxonomy1030014/s1>, Table S1: Classification of the Egyptian *Launaea* Cass. (1912–2016), Table S2: Taxa of *Launaea* Cass. and *Reichardia tingitana* L. Pau collected from 63 sites, Table S3: List of macromorphological characters and their character states recorded for the 156 specimens of *Launaea* Cass. and *Reichardia tingitana* L. Pau., Table S4: List of micromorphological characters and their character states recorded for the 156 specimens of *Launaea* Cass. and *Reichardia tingitana* L. Pau., Table S5: The characteristic features of seventeen hypothetical groups discriminated macromorphologically. Figure S1: Map of Egypt indicating the localities of the 63 sampling sites (Google Earth Pro 7.1.2.2041). Figure S2: *Reichardia tingitana* deposited in ALEX Herbarium. Figure S3: *Lactuca orientalis* (Syn. *Scariola orientalis*) deposited in ASTU Herbarium. Figure S4: *Launaea spinosa* deposited in CAI herbaria. Figure S5: *Launaea capitata* deposited in ALEX herbaria. Figure S6: *Launaea intybacea* deposited in CAI herbarium. Figure S7: *Launaea massauensis* deposited in CAI herbarium. Figure S8: *Launaea nudicaulis* deposited in ALEX herbarium. Figure S9: *Launaea angustifolia* subsp. *angustifolia* deposited in ASTU herbarium. Figure S10: *Launaea arabica* deposited in CAIM herbarium. Figure S11: *Launaea fragilis* subsp. *fragilis* deposited in ALEX herbarium. Figure S12: *Launaea mucronata* subsp. *cassiniana* deposited in CAIRC herbarium. Figure S13: *Launaea mucronata* subsp. *mucronata* deposited in CAIRC herbarium.

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