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Research Article

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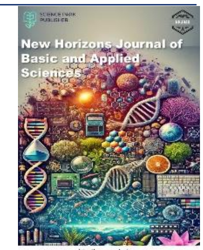

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Integrative taxonomy of selected Dictyotaceae from Hurghada, Egypt: An AI-assisted approach

Zeinab M. A. Mahfouz[1], Nehad M. Ibrahim[2], Dalia G. Gabr[1], Sameh B. Elkafrawy[3], El Sayed A.E. Hamed[4] and Rawheya A. Salah El Din[1,*]

[1] Botany & Microbiology Department, Faculty of Science, Al-Azhar University (Girls Branch) Cairo, Egypt, 11754

[2] Director of Software Development Department, Misbar Al-Kawn Information Technology Company, Cairo, Egypt

[3] Head of Marine Science Department, National Authority for Remote Sensing and Space Sciences (NARSS), Cairo Egypt

[4] Invertebrate Aquaculture Lab, National Institute of Oceanography and Fisheries (NIOF), Hurghada, Egypt

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Abstract

This study provides a taxonomic overview of selected brown algae belonging to the family Dictyotaceae (order Dictyotales, class Phaeophyceae) from the Red Sea coast at Hurghada. Species of *Dictyota* and *Padina* were examined based on key morphological characteristics, including thallus structure, holdfast type, branching pattern, blade morphology, and calcification. A diagnostic identification key was developed to facilitate species discrimination. A total of 47 qualitative and quantitative morphological characters were analyzed using PRIMER 6 (v6.1.6) to assess interspecific relationships through cluster analysis. In parallel, an artificial intelligence approach integrating convolutional neural network (CNN) feature extraction, image-based descriptors, dimensionality reduction, and clustering algorithms was applied to differentiate morphotypes. The combined use of numerical taxonomy and AI-based analysis enhances species identification and provides a robust framework for resolving taxonomic relationships among Dictyotaceae species.

Keywords: Dictyotaceae; Numerical taxonomy; Artificial intelligence; Morphological analysis; Cluster Analysis; Red Sea

1. Introduction

Macroalgae (seaweeds) form extensive and productive components of coastal ecosystems, where they stabilize sediments, reduce coastal erosion, and provide habitat and nursery grounds for diverse marine organisms [1]. They also contribute significantly to blue carbon sequestration [2]. The growth and distribution of marine macroalgae are closely associated with environmental conditions, particularly light intensity, temperature, nutrient availability, and salinity. Consequently, investigating their diversity is fundamental for understanding ecosystem processes and supporting effective conservation strategies [3].

Brown algae (class Phaeophyceae) constitute one of the major groups of marine macroalgae, comprising approximately 2,124 recognized species [4]. As dominant primary producers in many coastal environments, they contribute substantially to marine food webs and global carbon cycling [5]. Their classification was initially based on morphological and reproductive characteristics; however, advances in molecular phylogenetic studies have greatly refined the understanding of evolutionary relationships within the group [6, 7].

Among brown algae, the order Dictyotales is notable for its wide range of vegetative and reproductive features, many of which provide valuable characters for species identification and taxonomic classification [8]. Molecular evidence suggests that Dictyotales represent an early diverging lineage within

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Phaeophyceae [9]. The order includes the family Dictyotaceae, which is divided into two tribes, Dictyoteae and Zonarieae, based on the number of meristematic cells at the apices, and represents a dominant component of tropical and temperate marine floras [10].

Taxonomy provides the framework for naming, describing, and classifying organisms, forming the basis for studies of biodiversity, species distribution, and ecological relationships [11]. Morphological characteristics remain essential for species delimitation, supporting biodiversity assessment, conservation, and sustainable resource use.

This study investigates the taxonomy and morphological diversity of selected Dictyotales species from the Hurghada region of the Red Sea and develops a practical identification key supported by numerical and integrative approaches for accurate species discrimination and ecological assessment.

2. Materials and methods

2.1. Sampling sites

A total of eight specimens of brown algae (order Dictyotales) were collected from coastal areas in front of the National Institute of Oceanography and Fisheries (NIOF), Hurghada, Red Sea (27°17′07″ N, 33°46′21″ E), as shown in Figure 1. Sampling was conducted along rocky shores, coral reefs, and shallow subtidal zones. Specimens were collected during the summers of 2021-2023 at low tide by snorkeling to facilitate sampling.

2.2. Collection and preservation

Thalli were carefully detached using a knife or scissors, ensuring preservation of the holdfast whenever possible. Immediately after collection, specimens were rinsed with seawater to eliminate attached sediments and debris. They were subsequently preserved in a 4% formalin-seawater solution, while representative material was pressed and dried for herbarium preparation and morphological analysis. Each specimen was photographed, and its diagnostic morphological features were documented.

2.3. Identification of seaweed species

Species were identified by examining their morphological characteristics and comparing them with standard taxonomic keys and published references [12-15]. The classification of the order Dictyotales followed the system proposed by Silberfeld *et al.* [7]. Species names were checked against the AlgaeBase database to confirm currently accepted nomenclature, synonyms, and holotype information [4]. Brief diagnostic descriptions of genera and species were prepared. The collected specimens were identified using a dichotomous key constructed from distinctive morphological characters, including thallus form, blade shape, and branching pattern. The key was designed to support reliable identification under both field and laboratory conditions.

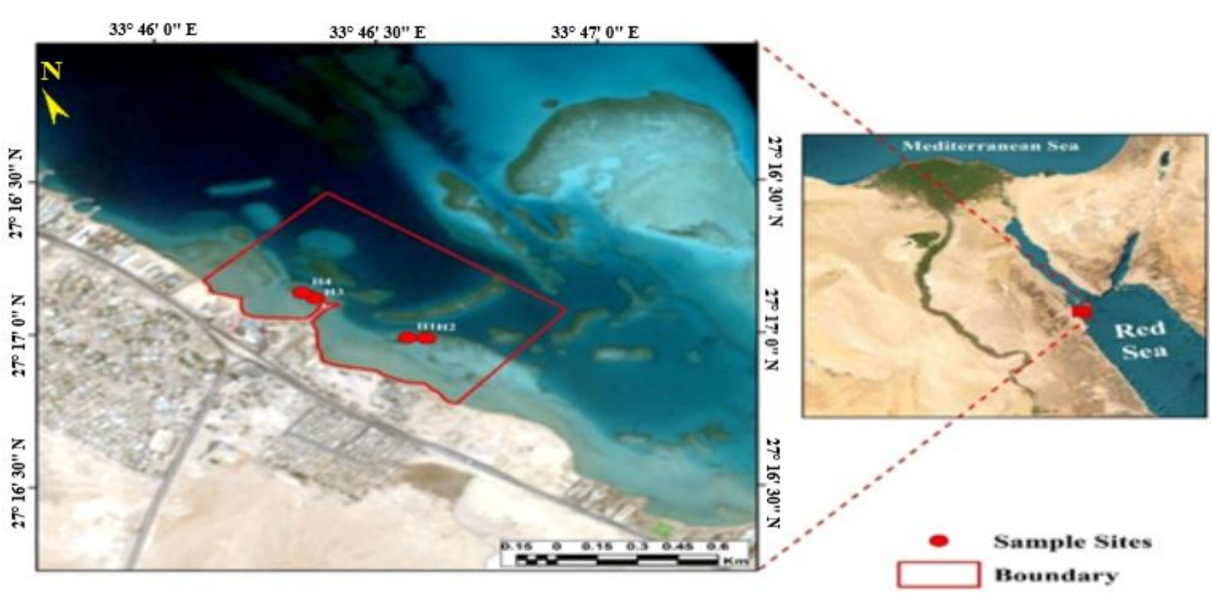

Figure 1. Map of the study area and sampling sites along the Red Sea coast, Egypt.

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2.4. Morphological analysis

Morphological characters recorded included thallus type, holdfast structure, stipe morphology, branching pattern, frond shape, apex, base, margin characteristics, and the presence or absence of calcification. Specimens were examined using a stereomicroscope (Seiwa Optical No. 620524, Correct Tokyo) and a light microscope (Olympus CX21 Binocular microscope). Measurements of length, width, and diameter were taken using a ruler.

2.5. Data compilation

Observations were compiled in tables summarizing key morphological features of each species. A total of 47 morphological characters for the studied species were used for numerical analysis. The relationships among the species were illustrated in the dendrogram, generated using the statistical program PRIMER 6 (version 6.1.6).

2.6. Artificial intelligence (AI) methods

The proposed AI approach combines deep CNN-based feature extraction, classical image descriptors, dimensionality reduction, and multiple clustering algorithms. The proposed clustering framework combines deep learning with conventional image analysis techniques to improve the discrimination of algal images. The workflow consisted of the following steps:

2.6.1. Image acquisition and pre-processing

All images were converted to the standard Red-Green-Blue (RGB) color format to ensure uniformity among samples, regardless of their original acquisition method or color depth.

2.6.2. Deep feature extraction

Image features were extracted using a pre-trained ResNet50 CNN available in the PyTorch torchvision library. To use the model as a feature extractor, the final fully connected classification layer was removed, retaining only the convolutional backbone. This configuration generated a 2048-dimensional feature vector that represented the high-level visual characteristics of each image.

2.6.3. Classical image feature descriptors

To complement the CNN's high-level features, three classical image descriptors were computed:

A. Dominant color extraction
B. Edge density
C. Aspect ratio

2.6.4. Feature scaling and dimensionality reduction

Due to the high dimensionality of the combined feature vectors, a two-stage dimensionality reduction process was applied.

2.6.5. Standardization

StandardScaler normalized all features to zero mean and unit variance, improving performance for principal component analysis (PCA), Uniform Manifold Approximation and Projection (UMAP), and distance-based clustering algorithms.

2.6.6. PCA

PCA serves two purposes:

A. Removes noise and correlations.
B. Provides a compact representation for UMAP and hierarchical clustering.

2.6.7. Hierarchical clustering and visualization

2.6.8. Dendrogram generation

Hierarchical clustering was performed using:

a. Linkage method: complete (maximum inter-cluster distance)
b. Labels: original filenames
c. Orientation: left-side vertical layout

2.6.9. PCA scatter plot

A scatter plot was generated using the first two PCA components, colored by KMeans cluster labels. This visualizes the global structure of the dataset and the separation between clusters.

A total of 40 images representing eight Dictyotaceae species were used for feature extraction and clustering analysis. The images were imported using OpenCV and resized to 224 × 224 pixels, corresponding to the input size required by the ResNet50 model. When necessary, grayscale images were automatically converted into three-channel RGB images. Image preprocessing was carried out using the standard preprocess_input() function available in TensorFlow/Keras for ImageNet-trained ResNet50 models. No image augmentation procedures were applied.

A pre-trained ResNet50 model (ImageNet weights, include_top = False) was employed exclusively as a feature extractor. Global average pooling was applied to the final convolutional layer, producing a 2048-dimensional feature

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vector for each image. These feature vectors were then analyzed using hierarchical agglomerative clustering with Ward linkage to evaluate the phenetic relationships among the investigated taxa. All analyses were performed in Python using TensorFlow/Keras, OpenCV, NumPy, SciPy, Matplotlib, and Scikit-learn.

Because ResNet50 was used only for feature extraction, no additional model training or fine-tuning was carried out. Consequently, parameters associated with model optimization, such as learning rate, optimizer, batch size, number of epochs, training accuracy, validation accuracy, and loss values, were not applicable. The extracted feature vectors were analyzed directly using hierarchical agglomerative clustering to examine relationships among the studied species.

2.6.10. AI limitations

Although the proposed approach produced reliable clustering results, several limitations should be considered. The analysis was based on a relatively small image dataset, and its performance may be influenced by image quality, specimen orientation, and lighting conditions. Furthermore, the model relies entirely on visible morphological features and therefore cannot distinguish cryptic species that require molecular evidence. Consequently, AI-based image analysis should be regarded as a complementary tool that supports, rather than replaces, conventional taxonomic expertise and molecular approaches.

3. Results and discussion

The taxonomic identity of the collected Dictyotales species was evaluated through detailed morphological examination and comparison with published taxonomic literature. A wide range of diagnostic characters was investigated, allowing reliable identification and taxonomic assessment of the recorded species.

A dichotomous identification key was prepared using the observed morphological characters, together with detailed species descriptions and original illustrations based on the examined specimens. Taxonomic identifications were verified by comparison with both classical and recent references. Combining field observations with published taxonomic information improved the reliability of species identification and provided a comprehensive assessment of the morphological diversity and variation of Dictyotales in the study area.

3.1. Taxonomic key for *Dictyota* and *Padina* (Family Dictyotaceae)

1. Thallus branched, strap-shaped or linear; not fan-shaped or calcified …...........................**Genus**: ***Dictyota*** ..…………**2**

1`. Thallus fan-shaped (flabellate) or funnel-shaped; often calcified…………………. Genus: ***Padina*** ………………..**4**

2. Branches wide up to 6 mm, margin of thallus dentate; thallus thick, leathery and crispy…………………***Dictyota ciliolata (D. ciliolata)***

2`. Branches slender, thin, flexible; generally flat entire margin and spirally twisted ………………………………... **3**

3. Branches (1-3 mm wide), interdichotomus 0.5-1 cm length, forked lobe with rounded apices……..……………..... ***Dictyota dichotoma (D. dichotoma)***

3`. Branches 0.5-1 mm wide and loosely branched; interdichotomous 0.5-2 cm length, the terminal apex acute…………………………..…. ***Dictyota indica* (*D. indica*)**

4. Thallus funnel-shaped (cups) with heavy calcification on the upper surface giving it a chalky white appearance.... …............. ***Padina pavonica (P. pavonica)***

4`. Thallus flabellate (fan-shaped) or circular to semicircular; calcification variable **5**

5. Thallus circular**;** moderately calcified on the upper (inner) surface (whitish) and lightly on the lower (outer) surface (light brown); sporangia and hairs in lower surface..... .... ***Padina boryana* (*P. boryana*)**

5`. Thallus splitting longitudinally into many lobes narrow or broad...**6**

6. Thallus lobes shallow, spathulate or wedge-shaped; sporangia grouped in sori in concentric dark lines in lower surface.......................... ***Padina boergesenii (P. boergesenii)***

6`. Thallus lobes deeply, fan shaped or wedge shape; calcification distinct between surfaces sporangia in both surfaces…....................................... **7**

7. Thallus thin and soft, fan-shaped lobes; lightly calcified on both surfaces.....................................***Padina tetrastromatica (P. tetrastromatica)***

7`. Thallus thick and strong; wedge-shaped lobes lightly calcified on the upper surface and moderately to heavily in lower surface...............................…***Padina gymnospora* (*P. gymnospora*)**

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3.2. Morphological notes on representative genera and species with classification

Class: Phaeophyceae

Subclass: Dictyotophycidae Silberfeld, F. Rousseau et Reviers, subclass. nov. prop.

Order: Dictyotales Bory de Saint-Vincent (1828)

Family: Dictyotaceae J.V. Lamouroux ex Dumortier (1822) [incl. Scoresbyellaceae Womersley (1987)]

Genus 1: *Dictyota* J.V. Lamouroux 1809b: 38, nom. cons.

Synonyms: *Dilophus* J. Agardh (1882); *Glossophora* J. Agardh (1882); *Glossophorella* Nizamuddin et Campbell (1995); *Pachydictyon* J. Agardh (1894).

The thallus is flattened, erect to repent, small, and slender, ranging in color from yellow-brown to dark brown. Branching is regular or irregularly dichotomous, forming ribbon-like fronds with smooth, dentate, crenulate, or ciliate margins. A distinct midrib is absent. Apices are obtuse, rounded, apiculate, or acute, and inconspicuous hairs may be present. Sporangia occur either solitarily or aggregated in sori scattered over both thallus surfaces. Superficial proliferations may be present or absent, and attachment is achieved by a single or multiple rhizoidal holdfasts. The taxonomic characterization was reviewed in accordance with the descriptions provided by [10, 16].

Species (Sp.) 1. *D. ciliolata* Sonder & KÜtzing , 1859 (Plate 1 A)

Holotype: *Dictyota dichotoma* (Hudson) J.V. Lamouroux

Synonyms: *Dictyota ciliate* J. Agardh, 1841

Dictyota maxima Zanardini, 1872

The thallus is erect, fan-shaped to semicircular in outline, reaching up to 13 cm in height, and yellowish-brown in coloration. It is thick, leathery, and somewhat crisp in texture. Branching is regular, isotomously dichotomous, with moderately wide branching angles reaching approximately 45°. The branches are broad, flattened, and strap-shaped, with smooth surfaces and a nearly uniform width along most of the thallus. They gradually become wider toward the apices. Branches measure 3-6 mm in width, and the segments between successive dichotomies are approximately 1 cm long. The margins are generally entire but become slightly dentate near the branch tips. Branches are characteristically emarginate, terminating in rounded, obtuse, or truncate apices. Surface proliferations are scarce. Sporangia occur solitarily and are scattered over both thallus surfaces. Attachment to the substrate is achieved by a dense stupose holdfast composed of numerous fine rhizoidal filaments.

Sp. 2. *D. dichotoma* (Hudson) J.V. Lamouroux, 1809 (Plate 1 B)

Holotype: *D. dichotoma* (Hudson) J.V. Lamouroux

Synonyms: *D. apiculata* J. Agardh,1894

D. dichotoma f. *elongate* (KÜtzing) Schiffner, 1933

The thallus is erect, olive- to yellowish-brown in color, bushy in habit, and reaches up to 12 cm in height. The basal region is flattened, forming a short stalk-like structure. Branching is predominantly regular and isotomously dichotomous, although occasional irregular branching may occur, with all branches arranged in a single plane. The branches divide dichotomously at narrow angles, usually not exceeding 25°. The thallus is flattened, slender, thin, flexible, and membranous, with a smooth surface. Branches are short and narrow, measuring 1-3 mm in width, and remain relatively uniform throughout the thallus.

They are commonly strap-shaped and slightly twisted. The segments between successive dichotomies are approximately 0.5-1 cm long. Terminal branches are emarginate, terminating in lobes with rounded apices. Margins are generally entire but may bear numerous small proliferations. Sporangia occur in scattered groups on both thallus surfaces. Attachment to the substrate is achieved by a small discoid holdfast composed of fibrous rhizoidal filaments.

Sp.3. *D. indica* Sonder & KÜtzing,1859 (Plate 1 C)

Holotype: *D. dichotoma* (Hudson) J.V. Lamouroux

Synonym: *D. indica* f. *torta* P.Crouan & H. Crouan,1878

The thallus is erect, bushy, and reaches up to 14 cm in height. It is very slender, sparsely branched, and exhibits a color gradient from yellowish-brown in the upper portions to dark brown toward the base. Branching is repeatedly and regularly isotomously dichotomous, producing thin, flattened, ribbon-like branches with long, linear inter-dichotomous segments. The branches are loosely arranged and spirally twisted, measuring approximately 0.5-1 mm in width. The segments between successive dichotomies are 0.5-2 cm long, and the branches divide at moderately wide angles of up to about 45°. Branches maintain a relatively uniform width throughout most of the thallus but gradually narrow toward the apices.

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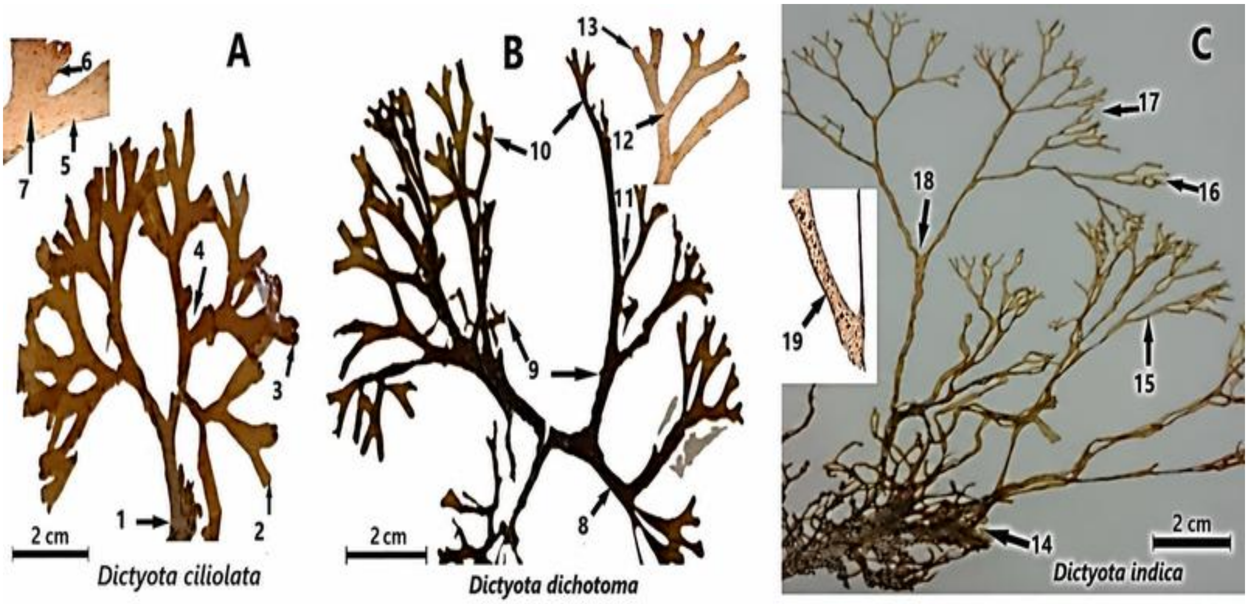

Plate 1. Morphological features of the thalli of **(A)** *Dictyota ciliolata*, **(B)** *D. dichotoma*, and **(C)** *D. indica*. **(1)** Stupose holdfast, **(2)** truncate apex, **(3)** rounded apex, **(4)** wide branching angle (45°), **(5)** entire margin, **(6)** dentate margin, **(7)** scattered sporangial sori, **(8)** entire margin, **(9)** small proliferations, **(10)** uniform branch width, **(11)** narrow branching angle (25°), **(12)** scattered sporangia, **(13)** rounded apex, **(14)** basal proliferations, **(15)** spirally twisted branches, **(16)** acute apex, **(17)** slender branches, **(18)** wide branching angle (45°), and **(19)** scattered groups of sporangia.

Margins are entire, and the terminal apices are acute. The thallus surface is smooth, lacking surface proliferations, although a few narrow proliferations may occur near the basal region. Sporangia are arranged in small scattered groups on both thallus surfaces. Attachment to the substrate is achieved by a discoid holdfast bearing rhizoidal filaments.

Genus 2*: Padina Adanson* 1763: 13, 586, nom. cons. 127

Synonym: *Dictyerpa* Collins et Harvey in Collins (1901); *Vaughaniella* Børgesen (1950)

The thallus is erect and supported by a short stipe, which may be partially covered by rhizoidal filaments. It consists of one to several flattened blades that are fan-shaped to funnel-shaped and may overlap. A distinct midrib is absent. Blades may be entire, divided, or irregularly eroded, with enrolled margins. Calcification varies in degree among specimens, forming a thin calcareous layer that imparts a brownish to whitish appearance to the thallus. Conspicuous, and occasionally faint, concentric bands of phaeophycean hairs are present on one or both blade surfaces. Reproductive structures occur as sporangia scattered in patches or arranged in sori, typically distributed in concentric zones between successive bands of hairs. The morphological characterization follows the descriptions of [16].

Sp.1. *P. pavonica* (Linnaeus) Thivy, 1960 (Plate 2 A)

Holotype: *P. pavonica* (Linnaeus) Thivy

Synonyms: *Zonaria pavonia* C.Agardh, 1820

Padina mediterranea Bory de Saint Vincent,1827

The thallus is erect, semicircular to circular, and forms funnel-shaped to fan-shaped fronds, reaching up to 7 cm in length. It is attached by a very short, compressed stipe-like base and exhibits a light to olive-brown coloration. The blades are broadly rounded, up to 8 cm wide, overlapping, and characterized by a rigid, thick, and leathery texture with strongly undulate margins. Calcification is pronounced, with the upper surface heavily encrusted by a thin calcareous and slimy layer, imparting a rough texture and a pale chalky whitish-brown appearance upon drying. The lower surface is moderately calcified. Fine concentric bands of phaeophycean hairs are conspicuous on both surfaces, alternating with concentric rows of dark sporangial sori. The sori are arranged in distinct concentric lines between successive hair bands on both blade surfaces. Attachment to the substrate is achieved by a bulbous holdfast bearing flexible, densely matted rhizoidal filaments.

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Sp.2. *P. boryana* Thivy, 1960 (Plate 2 B)

Holotype: *P. pavonica* (Linnaeus) Thivy

Synonyms: *Padina tenuis* Bory, 1827

Padina commersonii Bory, 1828

The thallus is erect, circular in outline, yellowish-brown in color, and reaches up to 6 cm in length. It consists of broadly flabellate (fan-shaped) to rounded blades measuring 4-6 cm in width. The blades are simple, with entire margins and a smooth surface. Calcification is moderate to strong on the upper (inner) surface, resulting in a rough texture and a pale chalky whitish-brown appearance when dry, whereas the lower (outer) surface is only lightly calcified and retains a light brown coloration. Reproductive structures are arranged in concentric rows of sporangial sori alternating with successive concentric bands of phaeophycean hairs, both of which are restricted to the lower (outer) blade surface. The thallus is attached to the substrate by a dense stupose holdfast composed of numerous rhizoidal filaments.

Sp.3. *P. boergesenii.* Allender &Kraft,1983 (Plate 3 A)

Holotype: *P. pavonica* (Linnaeus) Thivy

Synonym: *Padina tristromatica* Levring,1942

The thallus is erect, flabellate in habit, yellowish- to golden-brown in coloration, reaching up to 6 cm in height and 7 cm in width. The blades are longitudinally divided into numerous shallows, narrow to broad spatulate or wedge-shaped lobes, each measuring approximately 2-4 cm in length and 1-3 cm in width. Lobes possess attenuate bases, with margins and apices that are slightly undulate. The blades are thin, flattened, foliose, and somewhat brittle in texture. Calcification is weakly developed on both surfaces of the upper lobe regions, whereas the lower portions of the lobes are heavily calcified. Concentric bands of phaeophycean hairs are present on both blade surfaces and alternate with dark-brown concentric rows of sporangial sori. The sori are located immediately above the hair bands on the lower blade surface. Attachment to the substrate is achieved by a small bulbous holdfast covered with numerous fibrous rhizoids.

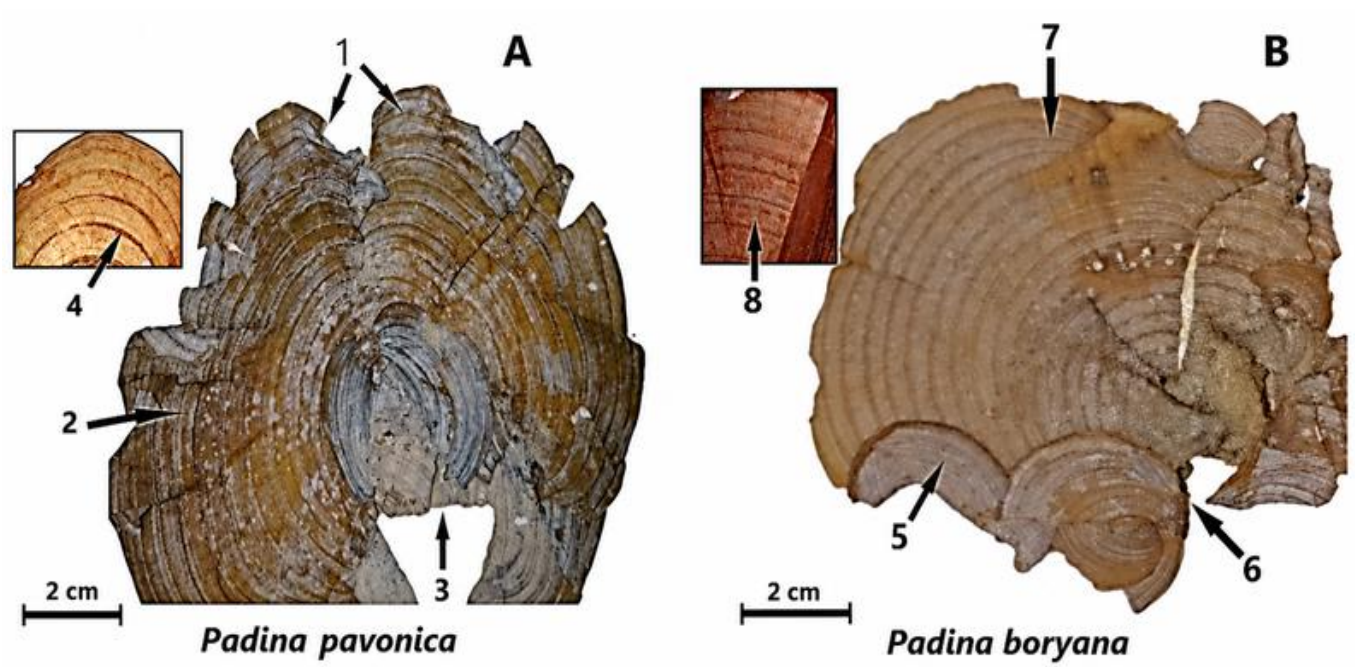

Plate 2. Morphological features of the thalli of **(A)** *Padina pavonica* and **(B)** *P. boryana*. **(1)** Thick, undulated blade, **(2)** heavy calcification with a thin slimy layer, **(3)** semicircular fan-shaped blade, **(4)** sporangial sori, **(5)** heavy calcification, **(6)** rounded blade, **(7)** light calcification, and **(8)** sporangial sori.

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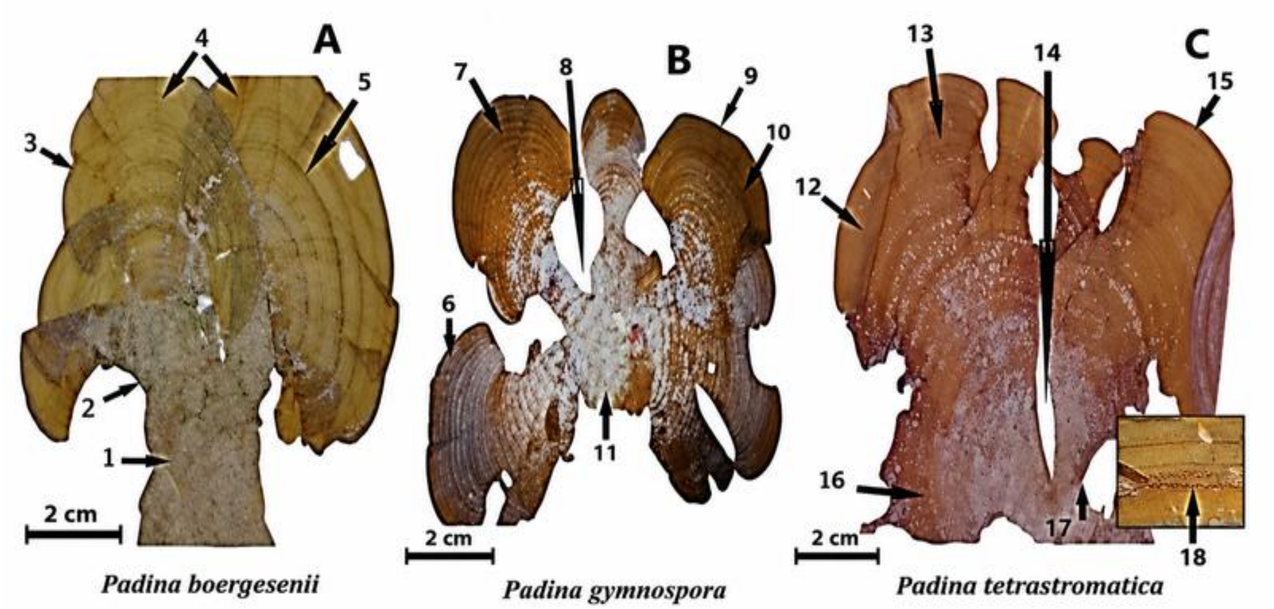

Plate 3. Morphological features of the thalli of **(A)** *Padina boergesenii*, **(B)** *P. gymnospora*, and **(C)** *P. tetrastromatica*. **(1)** Heavily calcified lower portion, **(2)** shallow, narrow spatulate lobe, **(3)** slightly undulated margin, **(4)** slightly calcified upper portion, **(5)** sporangial sori alternating with hairlines, **(6)** thick, robust lobe, **(7)** sporangial sori alternating with hairlines, **(8)** deeply divided wedge-shaped lobe, **(9)** slightly undulated margin, **(10)** lightly calcified upper portion, **(11)** heavily calcified lower portion, **(12)** thin, soft lobe, **(13)** slight calcification, **(14)** deeply divided fan-shaped lobe, **(15)** entire margin, **(16)** moderate calcification, **(17)** attenuate base, and **(18)** sporangial sori arranged in double rows.

Sp.4. *P. gymnospora* (KÜtzing) Sonder,1871 (Plate 3 B)

Holotype: *P. pavonica* (Linnaeus) Thivy

Synonyms: *Zonaria gymnospora* Kützing,1859

Padina vickersiae Hoyt,1920

The thallus is erect, flabellate (fan-shaped), thick and robust, reaching up to 7 cm in height and 8 cm in width. It is yellowish-brown in the upper portions, becoming dark brown toward the base. The blades possess entire, in-rolled margins that are slightly undulated and are deeply divided longitudinally into broad wedge-shaped to spatulate lobes measuring 1.5-3.5 cm in length and 1-2 cm in width. The blade surface is smooth throughout.

Calcification is slight on the upper surface but becomes moderate to heavy on the lower surface. Phaeophycean hair bands are inconspicuous and occur alternately on both blade surfaces. Dark sporangial sori are present on both surfaces, occurring either as solitary sporangia or as grouped concentric bands, and may also form discrete fertile zones between successive hair bands. The thallus is attached to the substrate by a small, short, solitary stupose holdfast.

Sp.5. *P. tetrastromatica* Hauck,1887 (Plate 3 C)

Holotype: *P. pavonica* (Linnaeus) Thivy

The thallus is erect, semicircular to flabellate (fan-shaped) in outline, reaching up to 8 cm in height and 7 cm in width. It is light brown in the upper regions, becoming dark brown toward the base. The blades are deeply divided longitudinally into fan-shaped lobes with in-rolled margins that are entire to slightly undulate in the upper portions. Each lobe possesses an attenuate base and remains connected to adjacent lobes at the basal region, measuring approximately 3-5 cm in length and 2-3 cm in width. The blades are flattened, thin, soft, and slightly leathery in texture, with smooth surfaces throughout. Calcification is weakly developed on both blade surfaces. Narrow concentric bands of phaeophycean hairs are present on both surfaces and alternate with concentric rows of sporangial sori. The sporangia are arranged in distinct double concentric lines encircling the hair bands on both sides of the blades. Attachment to the substrate is achieved by a small stupose holdfast composed of rhizoidal filaments.

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Table 1. Morphological characters used for numerical analysis of the investigated Dictyotaceae species.

No.	Characters		Codes
1	Growth habit		1. Solitary or clustered. 2. Clustered.
2	Thallus	Color	1. Golden brown. 2. Light brown. 3. Yellowish brown. 4. Olive brown.
3		Size	1. Large (more than 10 cm). 2. Small (less than 10 cm).
4		Strength	1. Thick rigid. 2. Slightly rigid. 3. Slender weak.
5		Regularity	1. Regular. 2. Irregular.
6		Shape	1. Flabellate, Funnel like. 2. Flattened straps, Semicircular out line.
7		Density	1. Bushy. 2. Bushy to sparse.
8	Holdfast	Shape	1. Discoid with rhizoids. 2. Stupose. 3. Rhizoids. 4. Bulb like with rhizoids
9	Stipe-like	Length	1. Short (equal or more than 10.mm). 2. Very short (less than 10 mm). 3. Not applicable.
10		Shape	1. Terete. 2. Flattened. 3. Compressed. 4. Terete to compressed. 5. Not applicable.
11	Branches	Existent	1. Existent. 2. Inexistent.
12		Type	1. Isotomous dichotomous. 2. Not applicable.
13		Pattern	1. Regular. 2. Regular to irregular. 3. Not applicable.
14		Length	1. Equal (uniform). 2. Unequal (slightly uniform). 3. Not applicable.
15		Direction	1. In one plane. 2. In all planes. 3. Not applicable.
16		Dichotomies	1. Close. 2. Widely spaced. 3. Not applicable.
17		Constriction	1. Uniform. 2. Constricted at intervals. 3. Not applicable.
18	Blades or Branches description	Type	1. Simple. 2. Divided or lobes.
19		length	1. Long (≥4 cm). 2. Medium (≥1.5 cm). 3. Small (≥0.5 cm).
20		Width	1. Broad (≥2 cm). 2. Medium (≥0.5 cm). 3. Narrow(<0.5cm).
21		Thickness	1. Thick, leathery. 2. Thin, membranous.
22		Overlapping	1. Present. 2. Absent.
23		Margin straightness	1. Entire, straight. 2. Undulate. 3. Twisted. 4. Slightly undulate.
24		Shape	1. Narrow, linear. 2. Strap like. 3. Funnel. 4. Flabellate, round (fan-shaped).
25		Lobing degree	1. Shallow to deeply lobed. 2.Deeply incised or divided. 3. Not applicable.
26		Lobes shape	1. Spathulate or wedge shape. 2. Fan shape. 3. Two equal straps. 4. Not applicable
27		Surface appearance	1. Slightly smooth. 2. Rough.
28		Surface zonation	1. Presence of concentric bands. 2. Absence of concentric bands.
29		Texture	1. Fragile. 2. Crispy 3. Slightly tough. 4. Chalky.
30		Base type	1. Slightly symmetrical. 2. Asymmetrical. 3. Not applicable.
31		Base shape	1. Cuneate. 2. Attenuate. 3. Not applicable.
32		Apex shape	1. In-rolled. 2. Emarginated, round apex of lobes. 3. Emarginated, obtuse and truncate apex of lobes. 4. Emarginated, acute apex of lobes.
33		Margin dentation and shape	1. Present, dentate. 2. Absent, entire.

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Table 2. (Continued).

34	**Sporangia**	**Aggregation**	1. Solitary. 2. In group(patches). 3. Solitary, in group.
35		**Position**	1. One surface. 2. Both surfaces.
36		**Arrangement**	1. Scattered. 2. Arranged in concentric rows.
37		**Location**	1. On surface, above hair lines. 2. On surface, both sides of hair lines. 3. Associated with hairs all over both surfaces.
38	**Hairs**	**Appearance**	1. Conspicuous. 2. Inconspicuous.
39		**Arrangement**	1. Scattered. 2. Arranged in lines.
40		**Position**	1. One surface. 2. Both surfaces.
41		**Aggregation**	1. Solitary or aggregated. 2. Aggregated in continuous lines.
42	**Sporangia Sori /Hairs arrangement**		1. Alternate bands. 2. Not applicable.
43	**Proliferation**		1. Present, many. 2. Present, low. 3. Absent.
44	**Calcification**	**Existence**	1. Existent. 2. Inexistent.
45		**Degree**	1. Heavily. 2. Moderate to slightly. 3. Slightly. 4. Not applicable.
46		**Exhibition**	1. In concentric zones. 2. Not applicable.
47		**Upper /Lower Surfaces**	1. Slightly/slightly. 2. Heavily or moderately/slightly. 3. Slightly/heavily. 4. Not applicable

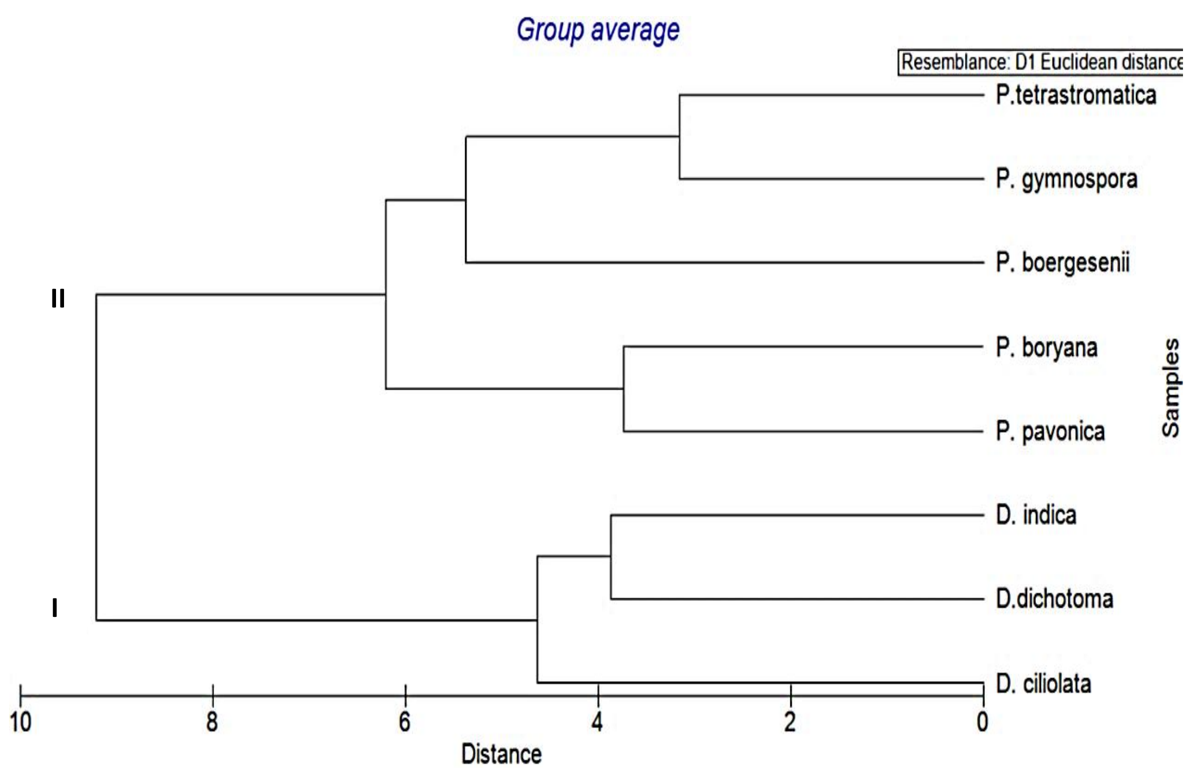

Figure 2. Hierarchical clustering dendrogram shows the relationships among eight Dictyotaceae species based on 47 morphological characters using PRIMER software.

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3.3. Numerical analysis of morphological characters

Numerical analysis of 47 morphological characters for eight species of Dictyotaceae (Table 1) was performed using PRIMER software based on Euclidean distance and group-average hierarchical clustering. The resulting dendrogram separated the investigated taxa into two major clusters corresponding to the genera *Dictyota* and *Padina* (Figure 2).

Cluster I comprised the three *Dictyota* species. *D. ciliolata* was clearly separated from *D. dichotoma* and *D. indica* by its broader strap-like thallus, crisp texture, low density of proliferations, stupose holdfast, and nearly dentate margins. These characters are consistent with the descriptions of [17-19], who reported that margin dentation in *D. ciliolata* may vary from dentate to completely smooth.

D. dichotoma and *D. indica* clustered together due to their narrow, flattened, membranous branches and discoid rhizoidal holdfasts. *D. dichotoma* differs from *D. indi*ca by having shorter, broader branches with rounded apices and abundant surface proliferations, whereas *D. indica* has longer, narrower branches with acute apices and proliferations mainly restricted to the basal region, consistent with the observations of Kyaw and Soe-Htun [20].

Although all examined species possessed scattered sporangia, reproductive structures are generally of limited taxonomic value in Dictyotales. Species delimitation within *Dictyota* remains challenging because of considerable morphological plasticity, with identification relying mainly on quantitative characters such as branching pattern, angles, inter-dichotomy length, and the presence of proliferations [18]. The present findings are consistent with recent taxonomic treatments of *D. dichotoma* and *D. ciliolata* [21, 22].

Cluster II comprised the five *P.* species. *Padina pavonica* and *P. boryana* clustered together owing to their broadly fan-shaped blades, cuneate bases, and strong calcification. They differed mainly in the distribution of sporangia, which occurred on both blade surfaces in *P. pavonica* but predominantly on the lower surface in *P. boryana*. The second subgroup comprised *P. boergesenii*, *P. gymnospora*, and *P. tetrastromatica*, all of which share longitudinally lobed blades, attenuate bases, and slight calcification. Among them, *P. boergesenii* is readily distinguished by its shallowly lobed blade, bulb-like holdfast, and sporangia confined to the lower blade surface. In contrast, *P. gymnospora* and *P. tetrastromatica* possess deeply divided blades, sporangial bands on both blade surfaces, and stupose rhizoidal holdfasts. The clustering pattern reflects the close taxonomic relationships within *Padina* and demonstrates the value of blade morphology, degree of calcification, and sporangial distribution as reliable diagnostic characters. As one of the few calcified genera of brown algae, *Padina* is recognized by its characteristic calcareous thallus, which remains an important taxonomic feature [23, 24]. Nevertheless, species delimitation within the genus is often difficult because of extensive morphological plasticity and overlap in diagnostic characters, particularly between *P. boergesenii* and *P. gymnospora* [25].

The morphological features observed in *P. pavonica* and *P. tetrastromatica*, especially the arrangement of sporangial sori in concentric bands, are consistent with previous descriptions. Likewise, the characters recorded for *P. boryana* correspond well with the description provided by Shabaka and Hosny [26]. The present observations also agree with Jothi and Selvi [27], who described *P. tetrastromatica* as having deeply divided blades, light calcification, and distinct zonation. These findings indicate that morphological characters continue to provide a reliable basis for species identification within *Padina*; however, the incorporation of molecular data is increasingly important for resolving taxonomic uncertainties [25, 28, 29].

The dendrogram further supports the separation of the genera *Dictyota* and *Padina*, emphasizing the taxonomic importance of blade morphology, branching pattern, degree of calcification, and reproductive structures in distinguishing members of the Dictyotaceae. The results demonstrate that morphological traits remain effective for distinguishing species and elucidating their taxonomic relationships. However, considerable morphological variability and environmental plasticity within both genera can complicate species identification and delimitation. Therefore, recent studies have emphasized the need to integrate morphological and molecular approaches to improve taxonomic resolution and achieve more accurate species delimitation within Dictyotaceae, and most useful for marine organisms [18, 23, 25, 28-30].

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3.4. AI-evaluation method

The internal validity of the clustering analysis was evaluated using the Silhouette Score, which produced a value of 0.70. This value indicates good cluster separation and satisfactory compactness among samples within the feature space. In unsupervised learning, a Silhouette Score above 0.70 is generally regarded as an indication of well-defined and reliable clustering. The score obtained in this study therefore indicates that the visual features extracted from the images adequately represent the morphological variation of the investigated species. It also suggests that the clustering procedure successfully retained the underlying structural relationships among the samples.

The hierarchical clustering dendrogram generated from ResNet50 image features (Figure 3) separated the eight investigated Dictyotaceae species into two distinct clusters corresponding to the genera *Dictyota* and *Padina*. The first cluster included the three *Dictyota* species (*D. indica*, *D. ciliolata*, and *D. dichotoma*), reflecting their overall similarity in thallus morphology and branching pattern. Within this group, *D. ciliolata* and *D. dichotoma* were more closely associated, whereas *D. indica* occupied a separate branch, probably because of differences in branch width, apical shape, and the occurrence of surface proliferations.

The second cluster contained the five *Padina* species and was further divided into two subgroups. One subgroup grouped *P. boryana* with *P. gymnospora*, indicating similarities in blade morphology and general thallus organization. The other subgroup included *P. boergesenii*, *P. pavonica*, and *P. tetrastromatica*, with *P. pavonica* showing the closest affinity to *P. tetrastromatica*. Their close association is likely related to similarities in blade shape, degree of calcification, and the arrangement of sporangial sori.

Overall, the dendrogram indicates that the deep features extracted by ResNet50 successfully captured morphological characters of taxonomic importance. The resulting clustering pattern was largely consistent with the traditional morphological classification. The clear separation between *Dictyota* and *Padina* supports their taxonomic distinction, whereas the internal subgroups reflect similarities and differences among species based on thallus morphology and reproductive characters.

The UMAP embedding (Figure 4) presents the distribution of the investigated species in a two-dimensional feature space generated from the extracted image features. Rather than forming a single compact cluster, the samples are distributed across the plot, indicating substantial morphological variation among the examined taxa. Several species occupy nearby positions, reflecting similar image features, whereas others are clearly separated, suggesting greater morphological divergence.

The distribution of the samples along both UMAP dimensions demonstrates that the extracted deep-learning features effectively distinguished among species while preserving their morphological relationships. The limited overlap between most groups indicates that these features possess good discriminatory ability. Consequently, the UMAP visualization supports the hierarchical clustering results and further demonstrates the suitability of the deep-learning approach for representing morphological similarities and differences within the Dictyotaceae.

Artificial intelligence (AI), machine learning, and particularly deep learning have become increasingly important in plant taxonomy by providing automated methods for species identification and classification based on morphological characters. These approaches complement traditional taxonomy by offering rapid, objective, and reproducible analyses that support biodiversity assessment and taxonomic research [31].

Recent developments in image recognition have promoted the use of RGB images for plant classification because they are inexpensive, readily available, and suitable for large-scale image acquisition. Among deep-learning methods, convolutional neural networks (CNNs) have proven especially effective for image classification and clustering owing to their ability to learn hierarchical image features automatically. Commonly used CNN architectures include AlexNet [32], VGG-16 [33], and ResNet [34].

CNN-based models have been successfully applied to plant identification, classification, and clustering in numerous studies [35, 36]. Clustering quality is commonly evaluated using the Silhouette Score, where higher values indicate greater cluster cohesion and better separation between groups [37]. More recently, deep-learning techniques, including CNNs, autoencoders, and generative adversarial networks

(GANs), have further improved image-based classification and clustering performance [38, 39].

In taxonomic studies, Ibrahim *et al*. [31, 40] demonstrated the usefulness of AI and transfer-learning models for plant identification and hierarchical clustering using images of leaves, fruits, and seeds. Their findings showed that deep-learning methods can effectively support species discrimination and taxonomic classification through automated image analysis.

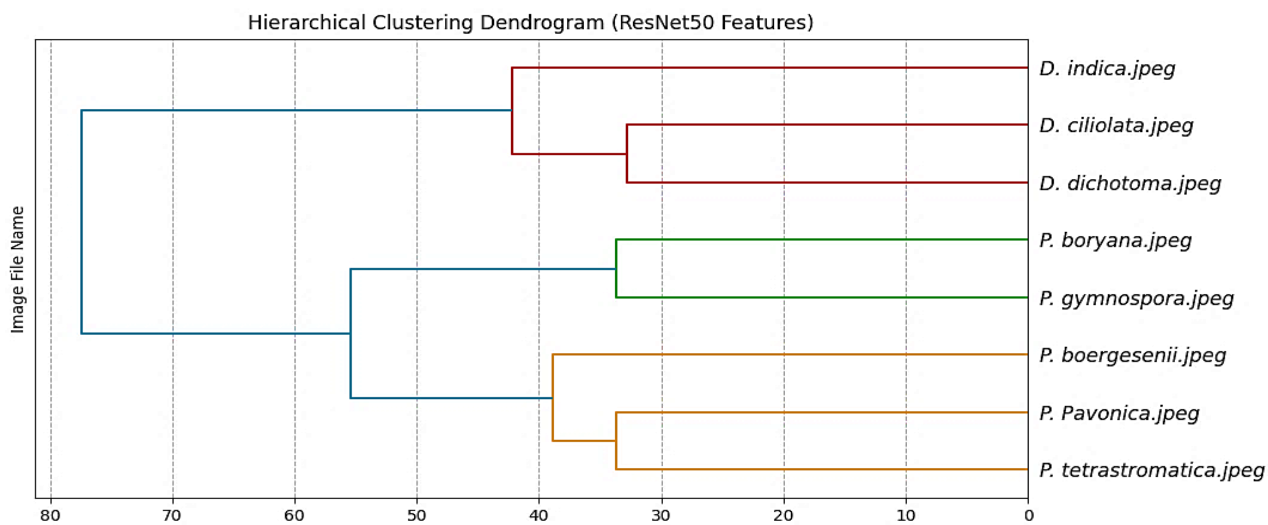

Figure 3. Hierarchical clustering dendrogram illustrating interspecific relationships and cluster merging patterns among eight Dictyotaceae species based on ResNet50 image features.

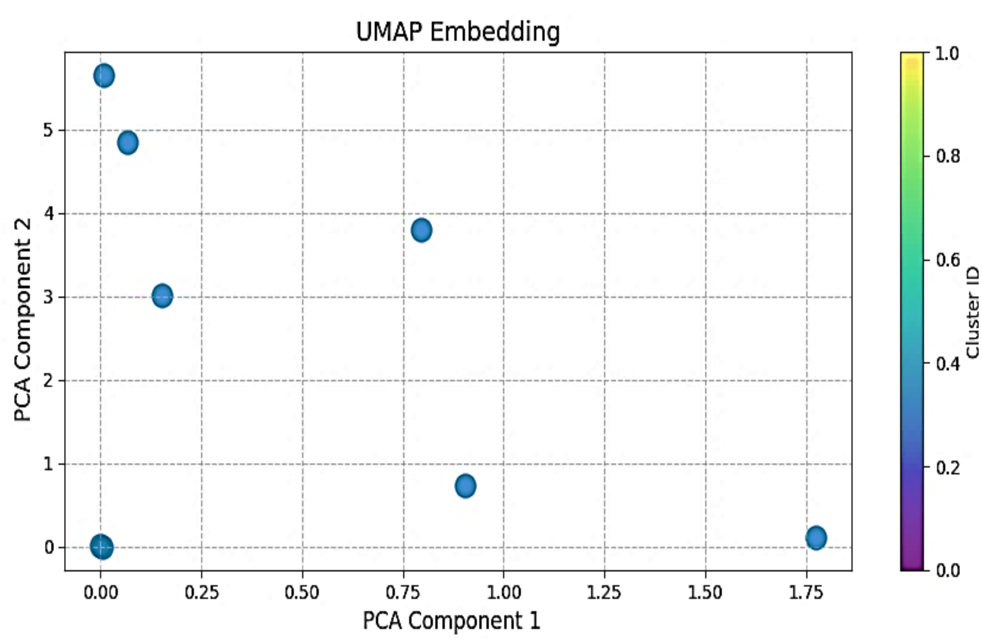

Figure 4. PCA scatter plot illustrating the distribution and relationships of eight Dictyotaceae species based on ResNet50-extracted image features.

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4. Conclusion

The present study demonstrated that combining traditional morphological taxonomy with artificial intelligence-based image analysis provides an effective approach for the classification of Dictyotaceae species. Numerical analysis of 47 morphological characters separated the investigated taxa into two principal clusters representing the genera *Dictyota* and *Padina*, confirming the taxonomic importance of branching pattern, blade morphology, holdfast type, calcification, and reproductive structures.

Within *Dictyota*, species separation was mainly associated with thallus architecture, branch dimensions, inter-dichotomy length, apical shape, and the presence of surface proliferations. In *Padina*, blade morphology, degree of calcification, and the distribution of sporangial sori were the most informative characters for distinguishing species. The clustering pattern was consistent with previous taxonomic studies and reflected both the similarities and differences among the investigated taxa.

The deep-learning analysis based on ResNet50 image features further supported the morphological classification. The hierarchical dendrogram together with the PCA and UMAP visualizations showed that the extracted image features successfully represented species-level variation and preserved meaningful taxonomic relationships. These findings demonstrate the value of CNNs as efficient tools for automated species identification and taxonomic research.

Although morphological characters remain fundamental for species identification, delimiting species within *Dictyota* and *Padina* is often complicated by morphological plasticity and overlapping diagnostic characters. Integrating morphological observations with deep-learning techniques and molecular data could therefore improve taxonomic resolution and provide more reliable species delimitation. Overall, this study demonstrates that combining classical taxonomy with artificial intelligence offers a robust framework for species identification, biodiversity assessment, and systematic studies of the Dictyotaceae.

Authors' contributions

The authors conducted the research and data analysis and were responsible for writing and approving the manuscript. All authors have thoroughly reviewed the work and provided their consent to the final version.

Conflict of interest statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author information

Corresponding authors: Rawheya A. Salah El Din*

E-mail: RawheyaSalahElDin1292.el@azhar.edu.eg

ORCID iD: 0000-0002-5596-2026

Data availability

Data will be available upon request.

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