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Bifurcation of Cryptocoryne Spiralis Species Based on Habitat Variations

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Abstract The subspecies or variety classification of Cryptocoryne spiralis is of great difficulty, caused by large variability in leaf and flower morphology. There is evidence in the literature on Cryptocoryne spiralis species that the extant description of the species is an amalgamation of two (or even more) different ecotypes, which is strengthened by reports of Allopolyploidy, and varying number of Chromosomes as reported by different cytologists, a rare phenomenon in the family Araceae. After extensive study of this species in its natural habitats of western India and cultivation, based on ecotypes, we propose to bifurcate this Species into two, Plaudis and Aquatica ecotypes based on observed significant differences in characteristics. Plaudis, Ephemeral, lacustrine and wetland ecotype which cannot thrive in Aquarium, does not grow well in any artificial conditions, has spurt growth pattern in torrential Konkan rain and becomes dormant in hot summers. Aquatica is riverine, rheophyte, perennial, aquatic ecotype, grows well in Aquarium, frequently flowering and fruiting, rapid germination of seeds and no observable dormancy period. These differences in traits are statistically significant at a p value of 0.05 (95% confidence interval). This study is morphological observations only and not on chromosome and DNA studies.

Keywords Cryptocoryne Spiralis, Cryptocoryne Retrospiralis, Taxonomy, Ecotypes

1. Introduction

The Araceae, also known as the arum family, is a large and diverse family of flowering plants with over 4,000 species in 140 genera. The family is found all over the world, except for the driest deserts and coldest regions. While most Araceae are terrestrial, there are a number of aquatic members of the family, found in a variety of habitats, including ponds, lakes, rivers, and streams.

One of the most well-known aquatic Araceae is the duckweed genus (Lemna). Duckweeds are small, floating plants with simple fronds (modified leaves) and flowers that are reduced to a single stamen and pistil. Duckweeds are found all over the world and are an important food source for many animals, including birds, fish, and invertebrates.

Another important aquatic Araceae genus is Cryptocoryne. Cryptocoryne are found in tropical and subtropical regions of Asia and Africa. They are typically rooted in the substrate and have a wide range of leaf shapes and sizes. Cryptocoryne are popular aquarium plants due to their attractive foliage and ease of care. Other aquatic Araceae include members of the genera Anubias, Bucephalandra, and Lagenandra. These plants are also found in tropical and subtropical regions of Asia and Africa. They are typically rooted in the substrate and have leathery leaves. These plants are also popular aquarium plants due to their attractive foliage and ease of care [1].

Aquatic Araceae play an important role in the ecosystems in which they live. They provide food and

shelter for a variety of animals, and they help to filter water and reduce pollution. Aquatic Araceae are also important cultural plants in some parts of the world. For example, duckweed is used as a food source and medicinal plant in many Asian countries. In addition to their ecological and cultural importance, aquatic Araceae are also popular ornamental plants. Many of the plants mentioned above are grown in aquariums and ponds around the world. Aquatic Araceae are relatively easy to care for and can add a touch of tropical beauty to any aquatic environment [2].

Cryptocoryne is a genus of aquatic plants from the family Araceae. It is the largest genus of aquatic plants in the world, with over 150 species. Cryptocoryne genus are native to tropical regions of Asia, including India, Southeast Asia, and New Guinea. They are typically found in slow-moving streams and rivers, as well as in seasonally inundated forests [3].

Cryptocoryne are popular aquarium plants due to their wide range of leaf shapes and colors. They are also relatively easy to care for, and can tolerate a variety of water conditions. However, they are sensitive to changes in the environment, and can melt their leaves if stressed [4].

All Cryptocoryne are aquatic plants, but some species are better adapted to living underwater than others. Some Cryptocoryne can even survive being completely submerged for extended periods of time.

The following Cryptocoryne species are particularly well-suited for aquatic life:

1. Cryptocoryne beckettii
2. Cryptocoryne parva
3. Cryptocoryne wendtii
4. Cryptocoryne lutea
5. Cryptocoryne balansea
6. Cryptocoryne crispatula
7. Cryptocoryne undulata
8. Cryptocoryne walkeri

These species are all native to tropical Asia, and grow best in warm water with moderate to low light. They are also relatively undemanding in terms of water chemistry, but prefer soft water with a pH of 7.0 or lower [4].

Cryptocorynes are popular aquarium plants because of their attractive appearance and ease of care. They can be used in a variety of aquarium layouts, from foreground plants to background plants. Cryptocorynes are also good for beginners, as they are relatively easy to grow and maintain.

The etymology of word Cryptocoryne, is “Crypto” meaning hidden or secretive, “Korune”, meaning club. These aquatic aroids have buried, or hidden, inconspicuous club like spadix, which are the inflorescence of family Araceae Juss (and Sub-family Aroideae Arnott) [5]. Like true Aroid, this species has buried, rhizomes which acts as photosynthetic energy, storage organ and help in avoiding the stressful summers of Indian subcontinent in dormancy. Many species are popular, aquarium plants grown for their beautiful dark green, bullous, leaves with undulate margins.

Cryptocoryne spiralis (Retzius) Fisch. ex Wydler (in 1830) is a native species of India, Bangladesh, and Sri Lanka. It is naturally found throughout the Indian subcontinent growing in rice fields, riverbanks, ditches, and other water bodies. They are slow-growing and a threatened species, due to invasive human activities, alien noxious weed infestation like Alternanthera philoxeroides (Mart.) Griseb. (Alligator weed) from South America more specifically from Argentina, Eichhornia crassipes (Martius) Solms (1883) (the common water hyacinth) from Brazil, Ipomoea carnea Jacq., (Gramophone flower bush), habitat destruction and water pollution [6].

1.1. Extent of Taxonomic Confusion

1.1.1. Economic Importance of Cryptocoryne Spiralis

Specimens originally exported from Maharashtra, are grown every day in nurseries and distributed to sellers. Cryptocoryne spiralis is a popular aquarium plant in the west and is easily cultivated in both emersed and submerged conditions. These plants traded in the aquarium hobby are perennial and continue to grow year-round in heated aquariums and occasionally flower or fruit in heated European and American aquatic plant nurseries [7].

Cryptocoryne spiralis is possibly the most common Cryptocoryne species encountered in India, occurring almost everywhere from agricultural land to mountainous streams. In addition, the rhizomes of Cryptocoryne spiralis have been used in Ayurvedic, Indian system of medicine for eons [8].

The rhizome extract of Cryptocoryne spiralis is used by the Ayurvedic physicians for the alleviation of diarrhea, cough, infantile vomiting, high fever and elevated bilirubin or jaundice. Local application of extract as poultice for healing skin burns and boils. Antibacterial activity of the rhizome extracts of Cryptocoryne spiralis and C. retrospiralis have been reported in literature. Even notorious myxomatous, flagellate bacteria like Pseudomonas aeruginosa and common Micrococcus aureus are found to be susceptible to rhizome extracts of C. spiralis. Cryptocoryne spiralis is known as “Nattativadayam” in Tamil Nadu and Nattativasa in Andhra Pradesh, it has been called “the East Indian Root” in the colonial times. Yoganarasimhan S.N and Shantha T.R [9] identified Cryptocoryne spiralis as source of Ayurvedic medicine “Granthika Tagara”.

1.1.2. Problem of Cryptocoryne Ecotypes

The following are the observations of aquatic plant experts regarding ecotypic and specific adaptations of Cryptocoryne spiralis. Karel Rataj and Thomas J. Horeman [11] in their popular book “AQUARIUM PLANTS their identification, cultivation and ecology”, considered to be a timeless, classic exposition among aquatic botanists, however, observes, from his experiments that Cryptocoryne spiralis is a terrestrial

species not an aquatic plant.

Rataj, K. [11] narrated the problem classification of Cryptocoryne is problematic as species identification requires spathe which are rare underwater. The following are the observations of Christel Kasselmann [6] in her book “Aquarium plants” states that the Cryptocoryne species exhibit high degree adaptation to a variety of specific environments and that special attention is needed in the description of individual species. For example, Cryptocoryne crispatula var. balansae is adapted to very hard water, thriving on sedimentary Limestone rocks in the mountains of Southern Thailand. Cryptocoryne ciliata is a helophytic species that is found in tidal zones, Cryptocoryne grabowskii is found growing in leaf peat substrate, Cryptocoryne bullosa (Sg. Sibiak) and Cryptocoryne keei is a Rheophytic species growing in fast running waters. Cryptocoryne exhibit a variety of characters adapted to specific environments. Many unique ecological adaptations of Cryptocorynes have not been well documented [12].

1.1.3. Observations on Existence of Distinct Cryptocoryne Spiralis Ecotypes Suspected

With regards to Cryptocoryne spiralis, she states that the acquired specimens had a limited capacity for underwater growth and did poorly in their aquarium. Prof. Cook [13] acquired new specimens from India, which grew well in the aquarium and easily reproduced in nursery. She observed that these specimens would become popular in the aquaristics [13].

Ostensibly, Prof. Cook [13] sourced his specimens from Konkan Pivavan; in the vicinity of location, this experiment was carried out. Arends, J. C., et al [7] state that these specimens had two different forms and that they grew and flowered quickly. However, there is no information on exact locality of acquisition of these specimens. This old suspicion of Prof. Cook of two forms cited in literature has been confirmed by this research.

Cryptocoryne spiralis are predominant in western part of India. Indeed, most of the existent western literature on Cryptocoryne spiralis is sourced from the specimens obtained in Western regions of Maharashtra state.

1.1.4. Allopolyploidy in Cryptocoryne Spiralis

A. K. Sarkar [14] described that Cryptocoryne spiralis has 2n= 90 chromosomes due to polyploidy, Allopolyploidy caused by Meiotic irregularities. Arends, J. C., et. al [7] reported that the chromosome number of Cryptocoryne spiralis is 2n= 33,66, 88 and 132, and their specimens were also sourced from Konkan region of Maharashtra, Savantwadi, Goa, Belgaum, Madokal and Pivavan. Dr Cook observed that the specimens were remarkable in fast growth and flowering. Haploidy, Diploidy and polyploidy seem to be common in this species. Heteroploidy is uncommon in Araceae family and is reported only in genera Biarum (2n=16,22,24,26,96) and Cryptocoryne. As stated by Engler in 1920 paper, there is a correlation between chromosome number and taxonomy. It is suspected that a lower number of Chromosomes are more evolved from higher numbers. This variation of reported chromosome number gives strength to the occurrence of more than one evolved ecotype and form within the species [14].

With regards to Cryptocoryne retrospiralis, Bastmeijer, J.D. [7,5] states that not much is known about this species though it was introduced more than 150 years ago. Cryptocoryne retrospiralis are morphologically difficult to distinguish from C. spiralis var. spiralis as both have similar, stiff leaves and that spathe is needed to settle the question of species. Indeed, Cryptocoryne species cannot be speciated without a detailed study of the Spathe which is rarely observed and is seasonal. Additionally, the Spathe is inconspicuous and hidden in the rosette of leaves which makes casual species differentiation impossible.

In my observations the species are easily distinguished by the common presence of long, straited, runners in Cr. retrospiralis, while Cr. spiralis rarely reproduces by runners, most of the time the superficial buds of horizontal rhizomes sprouting plantlets. Conversely, Cr. retrospiralis, especially those growing in water also produce plantlets along the rhizomes just like Cr. spiralis. Finally, the rhizomes of Cr. retrospiralis are dark, grayish in contrast to rhizomes of Cr. Spiralis, which are brownish. These two species were on occasion found even in close vicinity, for example, on eastern bank and western banks of Indryani river in Maval.

Cr. retrospiralis is even slower growing than Cr. spiralis. Cr. retrospiralis is seasonal plant, (like my description of Cr. Spiralis ecotype plaudis) growing in the rains, producing terete leaves (the chives form) in cold weather and finally dying out in hot summers. Entire clumps vanish in hot summers. Similar ephemeral growth characteristic from months November to January has been described for Cryptocoryne nevillii Hook. f. in Sri Lanka by Graaf D. and Arends J.C [15] in 1986.

In contrast, Cr. Spiralis var. spiralis is perennial plant, producing leaves throughout year, but morphology does vary with seasons. Red List Category and Criteria for Cr. retrospiralis is listed as Least Concern. Cr. spiralis is less threatened, since it is hardy species, less damaged by pollution and human invasion. Agricultural activities seem to affect Cr. retrospiralis to a larger extent [16].

1.1.5. Large, Variability of Leaf Morphology in Species of Cryptocoryne Spiralis

Cryptocoryne spiralis leaf morphology is very variable, all manner of narrow leaves, broader leaves, were produced depending on light and climate conditions. The Bogner's (2013) [16], classification of Cr. spiralis species based on leaves, length of Kettle and tail of the limb of the spathe into 1. C. spiralis: var. spiralis (exists) (Wydler 1830), 2.var. huegelii (questionable) (1855), 3.var. cognatoides (questionable) (1931) and 4.var. caudigera (doubtful).

In Figure 1, we showcase the remarkable morphological variations observed in Cryptocoryne spiralis var. Tiger, both in its submerged and emersed forms. These variations are characterized by light striations and brown leaves reminiscent of a tiger's coat pattern, a striking feature that becomes prominent when the plants are exposed to bright light and the leaves are allowed to float a few centimeters below the water's surface. Under normal light conditions, the same plant exhibits conventional leaf growth. Notably, this unique variation challenges the existing classification system of Cryptocoryne spiralis species, as it does not align with Bogner's [16] previous classification (2013:141-148), which primarily relies on leaf and kettle morphology, as well as the length of the kettle's tail. This discovery underscores the need for a more comprehensive understanding of Cryptocoryne spiralis var. Tiger and its place within the broader taxonomy of Cryptocoryne spiralis varieties.

Figure 1. Cryptocoryne spiralis var. Tiger Morphological Variations

My explanation is var. cognatoides is incorrectly identified Cryptocoryne cognatum (Yadav, S.R and Bogner [16]) which has habit overlap in Malvan region of Konkan. Both described plants have broad leaves and are found in same vicinity and habitats. The colour of spathe and leaf shape being very variable. The authors themselves state this as a source of confusion.

This classification is of no real value as Kettles/ Spathe were found of all length, and tail of limb of spathe was also variable, some in same plant and leaf morphology varied from season to season. Even the leaf colour was variable depending on light conditions and extent of submergence, fully underwater or just floating near the surface.

Rajakumar, T. J. S [19] described distinct morphology of Cryptocoryne tambraparaniana, as a new species from Tambraparni (is of Sanskrit etymology meaning: “the world of lotus”) river basin, a perennial river in state of Tirunelveli, Tamil Nadu, Southern India, which was later found to be variant of Cr. spiralis. From his research photographs, I can observe that this specimen was possibly Cr. spiralis ecotype aquatica.

Variable leaf colour is produced in response to variation in light conditions. Cr. spiralis var Tiger is a type of ecotype aquatica, and a popular household, aquarium plant in the USA and Europe [19].

2. Objective

To identify existence of distinct ecotype of Cryptocoryne spiralis collected from different non overlapping geographical habitats and to describe the differential features of the two plant ecotypes the perennial type “Aquatica” and ephemeral “Plaudis”.

3. Materials and Methods

To study the morphology, growth characteristics leaf production, flowering, and fruiting habits of Cryptocoryne spiralis obtained from various non-overlapping, geographically distinct habitats. This experiment was carried out over a period of 4 growing cycles of Cryptocoryne spiralis ecotype Plaudis. A total of 30 specimens were sourced from two ecological locations from rice fields, irrigation ditches, mountainous streams, and wetlands, to banks of perennial rivers and lakes were collected and studied in this research. Fully grown specimens which had produced spathe were collected to maintain homogeneity of the experiment.

A variety of soils were used for cultivation which included common garden red soil enriched with worm-casting, Amazonia light, ADA Nature Aquarium Aqua soil, Fluvial Stratum, Golden River sand and Tropica Aquarium soil.

Submerged cultivation with Carbon dioxide injection and Sobo LED type Aquarium light 21 Watts, (Model AL-480) was used. Exposing the Cryptocoryne spiralis to a minimum of 8 hours of Photoperiod a day divided into 2 photo-sessions with a 2-hour gap in-between in afternoon. Since this experiment was run over a long period of time, years on the end, the water and temperature parameters did vary.

The water parameters were Temperature ranged from 18 degrees centigrade in winter to 27 degrees centigrade in Summer, TDS meter readings of water used in the aquarium ranged from 30 ppm in rainy season to 80 ppm in the summer season. Actual water and climate parameters are expected to have little influence on the growth as these plants were sourced from native habitats within 5 to 100 kilometers from the actual place of my experiment.

3.1. Holotype Source

A total of 80 (40 of each ecotype) mature, plant specimens were collected from Sawarde, in Chiplun taluka, Ratnagiri district of Maharashtra state, which is located 61 KM landwards from District headquarters Ratnagiri. This locality belongs to Konkan, mountainous West costal division of the state. It is located South-west of 226 KM

from State capital city of Mumbai. Specimens were sourced from local rivers Krishna and Venna. Sawarde is placed with a GPS coordinate of 17.41 North and 73.54 East, Google code for this location is CG4Q+VGR. Additional specimens were collected from nearby locations namely:

1. 17.39 N and 73.55E, 9HR2+RQM Kasarwadi, Maharashtra
2. 17.38 N and 73.56E, 9HG4+5JR Hadkani, Maharashtra
3. 17.37 N and 73.55 E, 9G8X+JM8 Ambatkhol, Maharashtra
4. 17.17N and 73.65 E, 5JCX+7WJ Kule, Maharashtra
5. 16.95 N and 73.65 E, WMQ2+6W5 Salpe, Maharashtra
6. 16.88 N and 73.79 E, VQMP+J26 Khorninko, Maharashtra
7. 16.96 N and, 73.61 E, XJ57+RFM Dabhole Bk., Maharashtra

A total 80 mature plants were collected from various localities in the vicinity of Sawarde, these were divided into 2 groups of 40 plants from perennial lacustrine rivers, this set is denoted as Aquatica group "A" and 40 plants from seasonal ephemeral habitats like ponds, streams, and rice fields, this set is denoted by Plaudis group "B". Interestingly this was the location Cryptocoryne spiralis specimens were described for the first time and most of the extant western literature and Aquarium specimens are from this vicinity.

3.2. Species Description

Erect, unbranched growth, rice fields to waterside aquatic habitats, rooted in soil, absence of adventitious roots, leaf rosette, presence of a creeping rhizome and stoloniferous, grass-like leaves, sheath at the base of the leaves, prominent midrib, rosette habitus, unbranched, unbranched venation running till tip of the leaves. green to a bit reddish colored leaves are from 11-13 (15) cm long with a Petiole 5 – 6 (8) cm long, leaf sheath 2 – 3 cm long attached around the stems. leaf blade lanceolate, 6 – 7 (9) cm long and (0.3) 0.5 – 0.7 (0.9) cm wide, green, base is cuneate, and the leaf tips are finely pointed and sharp (cuspidate) [18].

Cryptocoryne spiralis species are identified by the unique appearance of their spathe, which is characteristic of each species of Cryptocoryne. It is reported that, a membranous septum is found occluding the kettle, which is uncommon in any other species of genus Cryptocoryne [4]. Cryptocoryne spiralis is described as floral spathe tube that is shorter than the limb of the spathe, bears a transversely lamellate limb and obconic tube. The classical description of Spathe as stated by Rataj, K [11] that purple red coloured, wrinkled, Spathe length varies from 10 to 25 cm long, with the limb usually up to 7.5 cm long, having spirally twisted body and horizontal ridges. s, and coarse warty inner side.

Inflorescence is spadix, which is characteristic of Araceae family, spathe, which is a leaf like bract is described up to 13 cm long, basal tubular portion is of 2 cm length and approximately 8 mm in diameter, the limb expands, lanceolate, acuminate, body is spirally twisted reaching to top, usually clockwise, from 6 to 11.5 cm long, light purplish to green purplish outside, dark purplish and with sub horizontal lamellations within the kettle as described by Dr. N Sasidharan [21], Kerala Forest Research Institute, Peechi.

4. Observations

4.1. Climatic Study of Cryptocoryne Spiralis

Konkan region receives an Average Annual rainfall 3482 mm. June and October months are seasons of torrential rains followed by cold winters. The growing season of many Cryptocoryne spiralis in natural habitat coincides with onset of rains and flowering and fruiting in winter. The months of July to October get heavy rains and this is the season of growth of Cryptocoryne spiralis in natural habitat, this is followed by Winter which triggers flowering and fruiting. The final stage is the hot Indian summer, which leads to dormancy of the plants, and discarding of the leaves and reemergence from seeds and Rhizomes in the next cycle [22].

Figure 2 illustrates the ecological characteristics of Sawarde. Cryptocoryne spiralis in and around the Sawarde region within Konkan. The map indicates the specific localities where specimens were collected for this study. Notably, Konkan province has historically been a prominent source of holotypes found in herbariums, including the valuable contribution of Dr. Cook's specimen. Furthermore, a substantial portion of extant Cryptocoryne spiralis nursery specimens in the western region originates from this locality, which significantly bolsters the significance of our research.

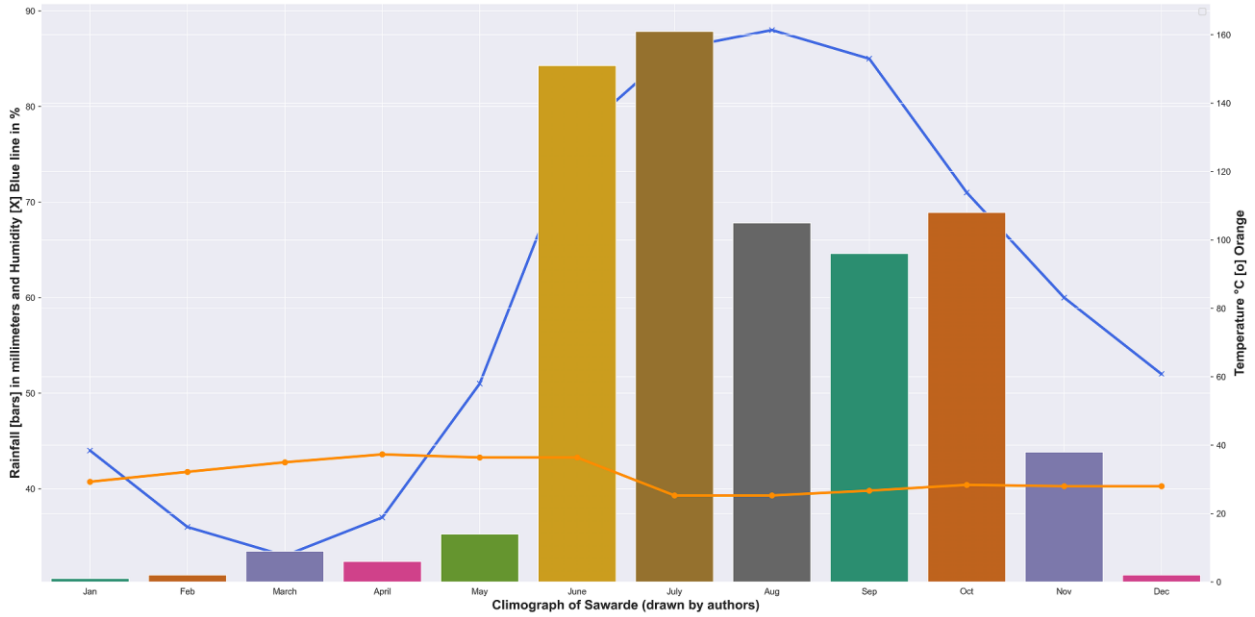

Figure 2. Ecological characteristics Cryptocoryne spiralis habitat- Climograph [22]

In addition, the annual Climograph of Sawarde is presented, providing a comprehensive view of the climatic variations experienced throughout the year. The blue line marked as "X" represents humidity percentages in the air, while the multicolored bars depict monthly rainfall in millimeters. The orange line marked as "O" showcases the mean monthly temperature. This Climograph aids in understanding the environmental factors that may influence the growth and distribution of Cryptocoryne spiralis in the region.

4.2. Observation on Leaf Length, Submerged Growth Characteristic, and Spathe Length

The fully mature leaf lengths of two species sets “A” and “B” were compared with each other. The mean leaf length of Aquatica “A” ecotype was 16.81cms (Variance 12.57) and Plaudis “B” ecotype was 13.53cms (Var 14.97). Shapiro-Wilk test for normality of data confirmed a Gaussian distribution at p value A= p=0.8433 and B =0.5491(both higher than 0.05), confirming the validity of our data collection. These two independent variables were compared with independent sample t test for statistical significance. The calculated p value of t test was 3.963 which shows statistically significant difference between the two ecotypes at a p-value of p= 0.0002 (98% significance).

In Figure 3, we present a graph displaying observations of leaf length and spathe length for two distinct groups: Group A, representing Aquatica, and Group B, representing Plaudis. To assess the significance of the differences between these groups, we conducted a statistical analysis using a Student's t-test. Our results revealed that the differences in leaf morphology between these groups were statistically significant, with a p-value. This finding highlights the meaningful distinction between Aquatica and Plaudis within the context of leaf length, providing valuable insights into the variability of these Cryptocoryne species.

The length of the Spathe was second variable measured and compared. The mean length of spathe of Aquatica ecotype was 7.03cms and Plaudis was 7.6cms. This was also tested by 2 sample independent t test. The calculated p value of t test was -0.642 and p-value=0.5239 (p>0.05) which means that there is NO appreciable difference between Spathe morphology.

Table 1 describes the comparative statistics and significance testing on leaf length and spathe length between the two Cryptocoryne ecotypes. For leaf length, the aquatica group showed a significantly higher mean and range compared to the paludis group. An independent two-sample t-test confirmed this was a statistically significant difference (p=0.0002). In contrast, for spathe length there was no significant difference between the groups, with similar means and variance. The t-test showed no statistical significance (p=0.5239).

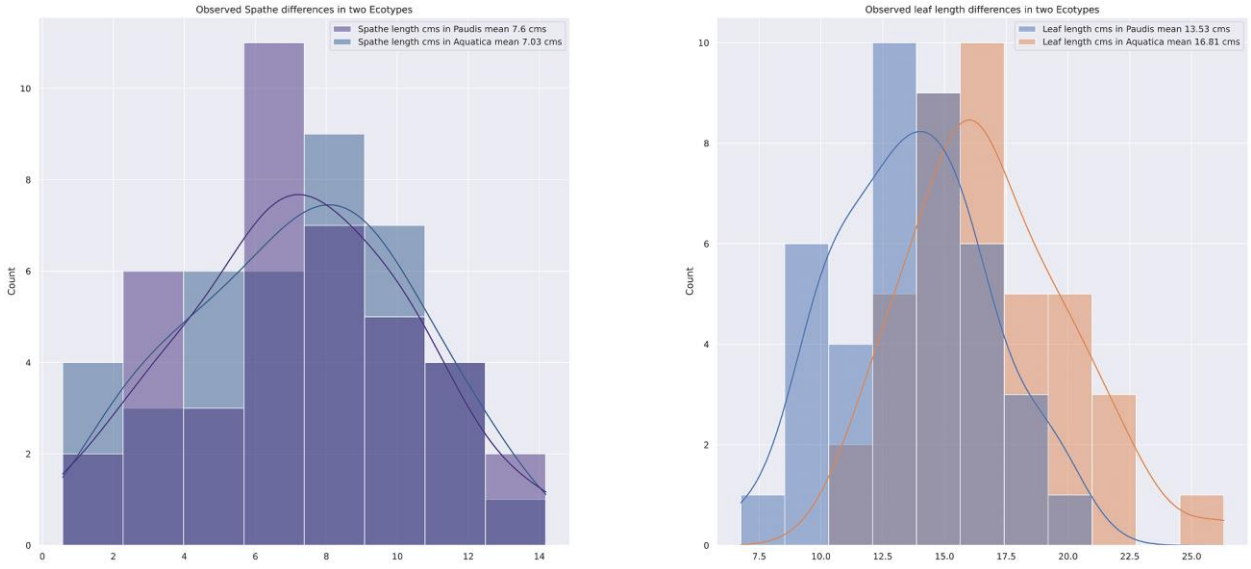

Figure 3. Leaf and Spathe Length Observations for Groups Aquatica and Plaudis

Table 1. Morphological Observations and Statistical Comparison between Groups A (Aquatica) and B (Plaudis)

Group A Aquatica Traits	Descriptive statistics	Group B Paludis Traits	Descriptive statistics	Statistical test of significance t-test Test static with P value and Interpretation of Significance level
Leaf length Group A Aquatica Shapiro-Wilk test p=0.8433 Gaussian distribution+	Specimens N = 40 Minimum and Maximum= (8.55, 24.6) Mean= 16.81 Variance =12.57 Skewness= -0.19 Kurtosis = -0.36	Leaf length Group B Aquatica Shapiro-Wilk test p=0.5491 Gaussian distribution+	Specimens N = 40 Minimum and Maximum= (3.58, 20.72) Mean= 13.53 Variance =14.97 Skewness= -0.37 Kurtosis = -0.12	Independent 2 sample t Test static= 3.963 and p value is =0.0002 **Leaf morphology** Statistically Significant difference between two ecotypes
Group A Aquatica Spathe length Shapiro-Wilk test p=0.0398 Not gaussian	Observations N = 27 Minimum and Maximum= (-2.99, 13.2) Mean= 7.03 Variance =13.08 Skewness= -0.88 Kurtosis = 0.96	Group B Aquatica Spathe length Shapiro-Wilk test p=0.7061 Gaussian distribution+	Observations N = 27, Minimum and Maximum= (2.15, 12.71) Mean= 7.6 Variance =7.57 Skewness= 0.02 Kurtosis = -0.78	Independent 2 sample t Test static= t is -0.642 and p value is =0.5239 Statistically NOT Significant. There is NO appreciable difference between Spathe morphology

Experiment on the underwater growth pattern of two groups in an aquarium was compared and analyzed. Carbon dioxide injection in the aquarium was used to enhance growth. Growth underwater was tested at 2nd week and 8th week, Submerged growth was significantly higher in Group A Aquatica compared to Group B Plaudis in both instances, Plaudis specimens remained stunted when submerged and did not grow properly. The difference between two groups was statistically very significant. Interestingly the total submerged growth of aquatic plants is almost always less than growth on emersed wetland, this is attributed to carbon dioxide limitation and reduction in light intensity underwater. The growth data was compared using independent t test. Calculated t value was 3.37696 and a p value of 0.00139 ($p<0.01$). The difference in growth underwater between the 2 ecotypes is 99% statistically significant. These observations is illustrated in figure 4.

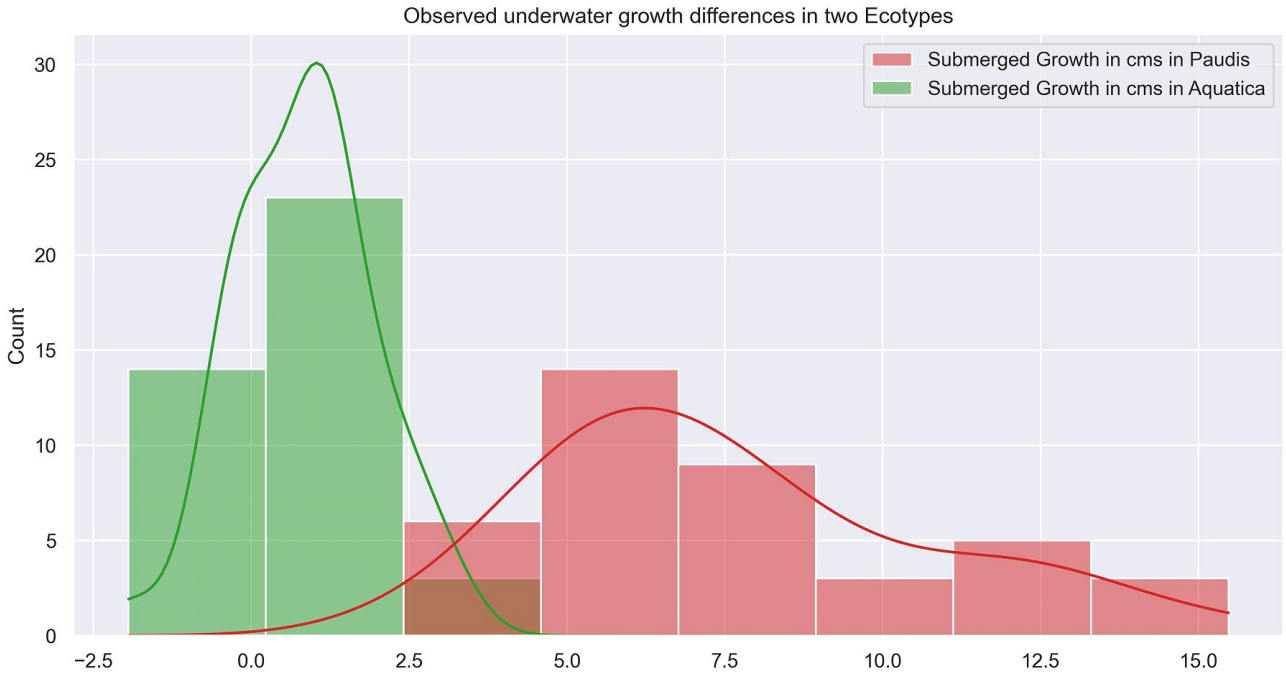

Figure 4. Significant difference in submerged growth between two groups

5. Discussion

Based on my observations of the characteristics of the various characteristics of Cryptocoryne spiralis, I propose for the first time in literature, classification of the extant species into two broad but distinct ecotypes.

This classification is very practical, useful in the field, based on the habitat of the plant and growing characteristics. This classification is not based on morphology of leaves or Inflorescence or dimensions of kettle which indistinguishable and practically of no use in the field. There is no observable difference in the Spathe borne in ecotypes, both are equally variable.

5.1. Distribution and Ecology of the Two Ecotypes-Ephemeral, Lacustrine "Paludis" Ecotype

The etymology of this word is derived from Latin, “Plaus” which means of the “of wetland” or “of marshland”. These are marshy, wetland Cryptocoryne spiralis. Their habitat are seasonal water bodies, transient wetlands, rice fields and hill steams. These wetland plants are never submerged in these situations, hence are not adapted to survival in aquarium. In addition, they are seasonal, that they die after flowering. As an adaptation to living in such stagnant conditions, these are very slow growing and do not produce copious leaves.

In Figure 5, we present a comparison of leaf morphology between the proposed two ecotypes of Cryptocoryne spiralis – "aquatica" and "plaudis." Notably, the "plaudis" ecotype is uniquely adapted to the seasonal life of wetlands, remaining consistently above water and, therefore, unable to thrive in aquarium environments. These plants enter a dormant state during the intensely hot Indian summers, a distinctive trait that sets them apart from the "aquatica" ecotype. This comparison sheds light on the ecologically significant differences between these two Cryptocoryne spiralis variants.

Figure 5. Leaf Morphology Comparison between Cryptocoryne spiralis Ecotypes – "Aquatica" and "Plaudis" [22]

They are difficult to grow artificially and do not thrive well on any soil. Few stunted, leaves are produced after a long, period of months of adaptation, flowering and fruiting are seasonal and common. Flower is spathe which lies half buried in soil and fruits are also buried underground. Specimens become dormant by culling leaves in response to hot, Indian summers. These cannot survive harsh winters. Finally, the stems are greener which seems to be noticeable difference between the two ecotypes [23].

5.2. Perennial, Riverine "Aquatica" Ecotype

The etymology of this classification is derived from

feminine gender of Latin “Aquatic” which means “of the water”. Their habitat are perennial waterbodies like large, riverbanks. These wetland plants are seasonally submerged in these situations, hence well adapted to survival in aquarium. As an adaptation to living in fast flowing waters, these plants are fast growing and produce copious leaves. Most of Cr. spiralis available in the west is of ecotype aquatica. As observed, these fit the profile of the aquatic variety of Cr. Spiralis [24].

In addition, they are perennial, that they do not die after flowering. They are very, easy to grow and thrive well on any soil/media. They can even be grown in full sunlight as potted plants. Adaptation is rapid, flowering is common. Flower is spathe which lies half buried in soil and fruits are also buried underground [26].

In Figure 6, we present a comparison of the flower morphology between the proposed two ecotypes of Cryptocoryne spiralis – "aquatica" and "plaudis." One notable observation is the sparse distribution of leaves on the "plaudis" ecotype when contrasted with the "aquatica" ecotype. This distinction in flower morphology highlights a key feature that contributes to the differentiation of these two Cryptocoryne spiralis variants.

Figure 6. Flower Morphology Comparison between Cryptocoryne spiralis Ecotypes – "Aquatica" and "Plaudis" [25]

Fruiting in cultivation is rare. There are very few fruit photographs on website, social media, and forums as these are ecotype “aquatica” as only var. spiralis seem to be available in the west. Interestingly, the Bastmeijer, J.D.’s [5] The Crypts pages website (Available from: http://crypts.home.xs4all.nl/Cryptocoryne/index.html) has no photographs of fruits of Cryptocoryne spiralis attests to this observation. This website quite famous among aquarium enthusiasts [26].

Specimens continue to grow after flowering and there is no dormancy. Presumably, these are cultivated in plant nurseries and greenhouses and can survive harsh European and American winters. Finally, the stems are coloured dark red which seems to be noticeable difference between the two ecotypes. The observed differences between the two are summarized in the given table [26].

In Figure 7, we provide visual representations of the flower, fruit, and leaf characteristics of the proposed ecotype of Cryptocoryne spiralis known as "aquatica." These large, hemispherical clumps of Cryptocoryne spiralis – "aquatica" are typically found thriving on the banks of rivers in Western India, particularly in Maharashtra. This ecological adaptation allows them to periodically submerge and, therefore, enables them to adapt to submerged conditions within aquariums [27]. The fruit of this ecotype is depicted as a berry with six locules, forming a syncarpium, and approximately six basally attached erect seeds per locule. The fruit wall exhibits thin septae with tubular radially oriented cells [29]. It is worth noting that the syncarp and fruit photograph included here are representative of the "plaudis" ecotype, as the "aquatica" ecotype infrequently produces fruits.

Figure 7. Depiction of Flower, Fruit, and Leaf of Proposed Ecotype "Aquatica" of Cryptocoryne spiralis [28]

The division of Cryptocoryne spiralis into two ecotypes: ecotype “aquatica” of perennial, riverine habitats, and ecotype “paludis" of ephemeral, lacustrine habitats, is based on years of my direct observations and cultivation of this species. This bifurcation is a fructuous one, where it is easy to classify the species in the field, based on simple observations of ecology, not in appearance of individual plants which show large variability [26].

As a summary the Table 2 provides a comparative feature summary of key ecological, morphological, and availability traits between the aquatica and paludis ecotypes. The aquatica form is adapted to perennial riverine habitats, exhibits better growth and flowering in cultivation, has variable spirally twisted spathes, and is readily available. The paludis ecotype is suited to seasonal stagnant waters, shows stunted growth in aquaria, has uniform spathes, and is unavailable in the West. In summary, the table highlights distinct ecological, morphological, and cultivation differences between these cryptic C. spiralis varieties.

Table 2. Comparative feature of the two ecotypes of Cryptocoryne spiralis

Name	
Ecotype *"aquatica"*	Ecotype *"paludis"*
Ecology	
Riverine Habitat- Moving perennial rivers and streams Perennial plant	Lacustrine Habitat-Seasonal, stagnant waters of ponds, rice fields, hill streams and dams Seasonal plant
Runners are rarely formed	Runners are never formed
Submerged growth is easy, augmented by Carbon dioxide injection	Not suitable for aquarium and no observed effect of Carbon dioxide injection on growth
Capable of growing in deep shade to partial shady banks, seems to prefer cooler parts, illumination of around 3000 lux, measured using a handheld illuminance meter or lux meter	Capable of growing in full sunlight to partial shade, seems to grow in full sunlight, like a terrestrial plant illumination of 40,000 lux, measured using a handheld illuminance meter or lux meter
Growth characteristics	
Grows albeit slowly, copious leaves Frequently flowering Fruiting in cultivation is rare, (Note: there are hardly any photographs of fruit among aquarists, on social media forums etc. *var. spiralis*) Year around growth No dormancy Slow and steady growth	Few, stunted leaves after long periods of adaptation, typically months Frequently flowering and fruiting in seasons Seasonal growth with periods of dormancy in summer, leading to occasional specimen culling all its leaves Very slow growth and in spurts
Morphology	
Leaves variable but smaller in size Undulated leaf margin and Narrow leaves in older specimens as adaptation to flowing waters Kettle variable but as described of this species Common, spirally, twisted, spathe Size variable Some clumps have dark, red colour of the stems	Leaves less variable, maybe larger in size in nature (see var. *cognatoides)* Kettle less variable but as described of this species Size less variable but comparatively bigger, sized specimens in nature Stunted in artificial cultivation Uniform, green colour of the stems
Availability in the western world	
Readily available in the west, thrives and hardy, most literature descriptions are of ecotype "aquatica"	Not available in west as does not thrive outside the adapted habitat, not much formal literature is available on ecotype *"plaudis"*
Status of the earlier Bogner (2013:141-148) classification	
Includes *Cr. spiralis* var. *spiralis* and var. *caudigera* and some other newer varieties like *Cr. spiralis* var. *tiger*	Unknown maybe includes *Cr. spiralis* var. *cognatoides*,

6. Conclusions

There is strong evidence that species Cryptocoryne spiralis is an amalgamation of many ecotypes and subspecies. This is due to the large geographical distribution of Cryptocoryne spiralis across the Indian subcontinent. Arguably, India offers a variety of environments for adaptation and evolution of the Cryptocoryne spiralis. This has been suspected by many researchers before and this is strengthened by chromosomal evidence of Aneuploidy, a rare phenomenon in the Araceae family. Observations of varying morphological features of Cryptocoryne spiralis and underwater growth characteristics were studied in 80 specimens obtained from natural habitats.

We propose to bifurcate this Species into two, Plaudis and Aquatica based on observed significant differences in characteristics. Plaudis, Ephemeral, lacustrine and wetland ecotype which cannot thrive in Aquarium, does not grow well in any artificial conditions, has spurt growth in torrential Konkan rain and becomes dormancy period in hot summers.

Aquatica is riverine, rheophyte, perennial, aquatic ecotypes, grows well in Aquarium, frequently flowering and fruiting, rapid germination of seeds and no observable dormancy period. The leaves of Aquatica are longer in size and stems are coloured red. Exposition of subspecies and ecotypes of Cryptocoryne can be better done using

Phylogenetic analysis, which is the scope of future research.

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