Chapter 1: Introduction

1.1. Lamiales **Bromhead (Mag. Nat. Hist. 2: 210. 1838.)**

Lamiales is one of the most diverse angiospermic plant order with approximately 21,878 species under 1,041 genera (Olmstead 2021). The order, Lamiales comprises 24 angiospermic families, under Eudicots-Asterids, according to the latest APG IV (2016) classification, namely, Acanthaceae, Bignoniaceae, Byblidaceae, Calceolariaceae, Carlemanniaceae, Gesneriaceae, Lamiaceae, Lentibulariaceae, Linderniaceae, Martyniaceae, Mazaceae, Paulowniaceae, Pedaliaceae, Phrymaceae, Plantaginaceae, Plocospermataceae, Oleaceae, Orobanchaceae, Schlegeliaceae, Scrophulariaceae, Stilbaceae, Tetrachondraceae, Thomandersiaceae, and Verbenaceae. They exhibit wide range of habitats, including aquatic to semi-aquatic (Lentibulariaceae, Linderniaceae, etc.), carnivorous (Byblidaceae, Lentibulariaceae), epiphytic (some Gesneriaceae), parasitic (Orobanchaceae), terrestrial (most members), etc. Liu *et al*. (2019) added Wightiaceae as a new monotypic family within the ever-resolving order Lamiales. Earlier versions of APG classification recognized Lamiales with 22, 21 (excluding Avicenniaceae, Buddlejaceae, Cyclocheilaceae, and Myoporaceae, by merging with Acanthaceae, Scrophulariaceae, Orobanchaceae, and Scrophulariaceae, respectively, and the addition of Calceolariaceae, Carlemanniaceae, and Plocospermataceae), and 23 (addition of Linderniaceae and Thomandersiaceae) families in APG I (1998), APG II (2003) and APG III (2009), respectively.

1.2. Linderniaceae **Borsch, Kai Müll. & Eb.Fisch. (2013)**

One of the phylogenetically most interesting families of Lamiales is Scrophulariaceae s.l. Several changes have been made to the familial circumscription of this polyphyletic family and several smaller families have been separated. Linderniaceae is one of the recently split out families from Scrophulariaceae s.l. (Rahmanzadeh *et al*. 2005).

The familial name is based on *Lindernia* All., a genus named after the pre-Linnean German botanist, F. B. von Lindern (Philcox 1965). Linderniaceae consists of approximately 250 species under 23 genera with tropical, sub-tropical and temperate distribution, distinguished by club-shaped staminal appendages (Table 1) (Stevens 2001, Rahmanzadeh *et al*. 2005, Fischer *et al*. 2013, POWO 2024).

Some Linderniaceae members, like, *Craterostigma plantagineum* Hochst.(native to Africa), *Chamaegigas intrepidus* Dinter (Native to Namibia, Africa), and *Linderniella brevidens* (Skan) Eb.Fisch., Schäferh. & Kai Müll. (native to Kenya and Tanzania) are well known poikilothermic plants (Fischer *et al*. 2013, POWO 2024).

Recently, Fischer *et al*. (2023) reported a possibly carnivorous new species of *Crepidorhopalon, C. droseroides* Eb.Fisch., Wursten & I.darbysh., from Mozambique. The plant is covered by sticky, glandular hairs, and three different arthropods have been reported to be stuck to the glandular hairs, from different herbarium collections. This increases the possible carnivorous members of Linderniaceae, from one to two. Earlier, *Vandellia cleistandra* (W.R.Barker) Eb.Fisch., Schäferh. & Kai was reported with the possibility of carnivory.

Torenia fournieri Linden ex E.Fourn. is widely used as an ornamental plant. *Legazpia polygonoides* (Benth.) T. Yamaz., *Lindernia procumbens* (Krock.) Philcox, *Torenia* *thouarsii* (Cham. & Schltdl.) Kuntze, *T. travancorica* Gamble, *Vandellia anagallis* (Burm. f.) T. Yamaz., etc. are medicinally important (Khare 2009).

Table 1. Worldwide distribution of the Linderniaceae genera.

Genus	**Number of species**	**Distribution**	**Source**
Ameroglossum Eb.Fisch., S.Vogel & A.V.Lopes	09	North-eastern Brazil	Almeidia *et al*. 2019, 2021, POWO 2024
Artanema D.Don	03	Tropical and sub-tropical old World	POWO 2024
Bampsia Lisowski & Mielcarek	02	S. Democratic Republic Congo	POWO 2024
Bonnaya Link & Otto	16	Tropical Africa, Madagascar, Tropical and Subtropical Asia, N. Australia	POWO 2024
Catimbaua L.P.Félix, Christenh. & E.M.Almeida	01	North-eastern Brazil	POWO 2024
Chamaegigas intrepidus Dinter ex Heil	01	Namibia	POWO 2024
Craterostigma Hochst.	27	Africa, Asia	POWO 2024
Crepidorhopalon Eb.Fisch.	33	Africa, Madagascar	POWO 2024
Cubitanthus Barringer	01	North-eastern Brazil	POWO 2024
Hartliella Eb.Fisch.	05	S. Democratic Republic Congo	POWO 2024
Hemiarrhena Benth.	01	Australia	POWO 2024
Isabelcristinia L.P.Felix, Christenh.&E.M.Almeida	01	Brazil Northeast	POWO 2024
Lindernia All.	66	Tropics and subtropics	POWO 2024

Linderniella Eb. Fisch.,Schaferh.&Kai Mull	16	Tropical and S. Africa, Madagascar.	POWO 2024
Micranthemum Michx.	13	U.S.A. to tropical and Subtropical America.	POWO 2024
Picria Lour.	01	South and Southeast Asia	POWO 2024
Pierranthus Bonati	01	Indo-China	POWO 2024
Schizotorenia T.Yamaz.	02	Indo-China to Peninsula Malayasia	POWO 2024
Scolophyllum T.Yamaz	03	Indo-China	POWO 2024
Stemodiopsis Engl.	07	Tropical and S. Africa	POWO 2024
Torenia L.	69	Tropics and subtropics	POWO 2024
Vandellia L.	52	Tropics and subtropics	POWO 2024
Yamazakia W.R.Barker, Y.S.liang &Wannan	02	Asia to N. Australia	POWO 2024

1.3. Systematic position of Linderniaceae

Bentham (1846) first treated today's Linderniaceae, as a distinct group and treated it as a sub-tribe, Lindernieae, under Gratioleae of Scrophulariaceae. He grouped together, *Artanema*, *Curanga*, *Torenia*, *Vandellia*, *Lindernia*, *Ilysanthes*, *Bonnaya*, *Pepidium*, and *Micranthemum*. Rahmanzadeh *et al*. (2005) first established Linderniaceae as a distinct family from Scrophulariaceae, under Lamiales. Rahmanzadeh *et al*. (2005) distinguished Linderniaceae based on the geniculate filaments (Fig 1).

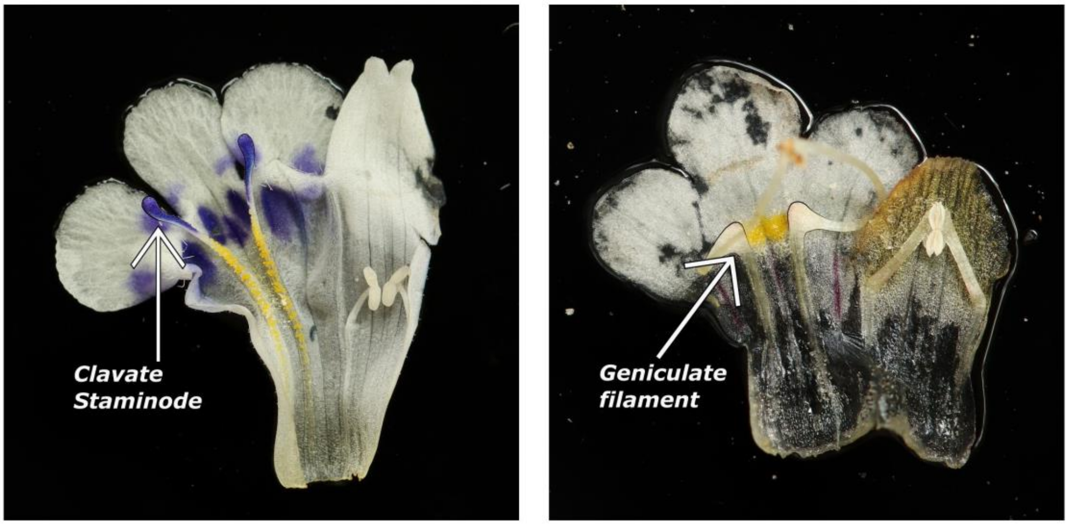

Figure 1. Clavate staminodes and geniculate stamens of Linderniaceae.

Then, Linderniaceae first appears in APG III (2009) classification as a distinct group. Fischer *et al*. (2013) further resolved the generic circumscription within Linderniaceae. APG IV (2016) placed Linderniaceae under Lamiales. Biffin *et al*. (2018) again tried to re-evaluate the systemic positions of a few more Australian *Torenia* and a few *Vandellia*, resulting in the establishment of a new genus, *Yamazakia*.

1.4. Diagnosis

These plants are distinguishable from the other related plant families based on the combination of 4-angular stem, estipulate opposite leaves, pinnate or palmate leaf venation, tubular or deeply lobed calyx, bi-lipped, tubular to campanulate corolla, smaller upper corolla lip, larger lower corolla lip, didynamaous stamens, jointed at apices, 2 shorter upper stamens, 2 longer lower stamens (sometimes appendaged or sometimes reduced to staminodes), spatulate stigma, poricidal or septicidal capsules, and small, aulacospermous or bothrospermous seeds (Fig 2) (Fischer *et al*. 2013, Stajsic 2022). *Ameroglossum* and *Stemodiopsis* exhibit the presence of bracteoles.

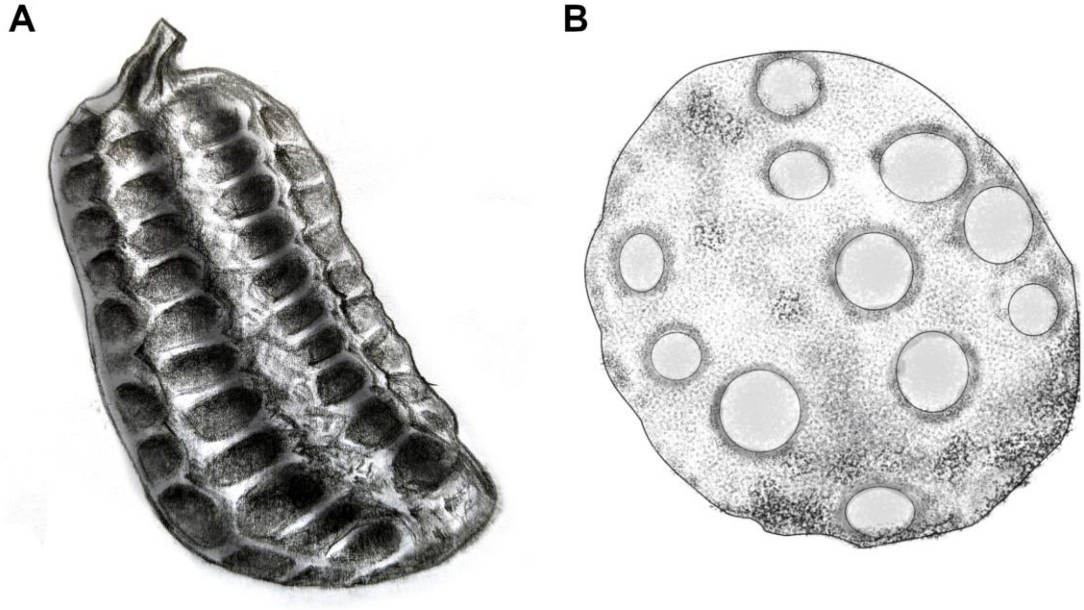

Figure 2. Illustration of two main seeds types. A, aulacospermous seed; and B, bothrospermous seed. (Fig. 2A courtesy: Ms. Rajesswary Goswami)

1.5. Distribution

Linderniaceae occupies a diverse type of habitats and landscapes, even a few genera and species are restricted to a particular region (Table 2) (POWO 2024). They vary from solely herbaceous in most members to basally woody, sub-shrubs, in *Ameroglossum* and *Stemodiopsis* (Balkwill 2020, Almeida *et al*. 2021).

Nine Linderniaceae genera are found within Indian limits, *viz.* ***Artanema*** D.Don, ***Bonnaya*** Link & Otto, ***Craterostigma*** Hochst., ***Legazpia*** Blanco, ***Lindernia*** All., ***Picria*** Lour., ***Torenia*** L., ***Vandellia*** L. & ***Yamazakia*** W. R. Barker, Y. S. Liang & Wannan (POWO 2024).

Table 2. Worldwide endemic genera and their distribution

Genera	Region
Bampsia Lisowski & Mielcarek	Democratic Republic of Congo
Catimbaua L.P.Félix, Christenh. & E.M.Almeida	NE Brazil
Stemodiopsis Engl.	Namibia
Crepidorhopalon Eb.Fisch.	Africa and Madagascar
Hartliella Eb.Fisch.	Democratic Republic of Congo
Hemiarrhena Benth.	North Australia
Isabelcristinia L.P.Félix, Christenh. & E.M.Almeida	NE Brazil
Linderniella Eb.Fisch., Schäferh. & Kai Müll.	Africa and Madagascar
Micranthemum Michx.	North and South America
Picria Lour.	Southeast Asia
Pierranthus Bonati	Cambodia, Thailand, Vietnam
Schizotorenia T.Yamaz.	Malaysia, Thailand, Vietnam
Scolophyllum T.Yamaz.	Cambodia, Laos, Thailand, Vietnam
Stemodiopsis Engl.	Africa

1.6. Morphology

Fischer (2013), based on phylogenetic & phenetic approaches produced more apparent circumstances for the generic treatments in Linderniaceae, along with providing a key to distinguish major genera. Almeida *et al*. (2021) provided morphological circumference for *Ameroglossum*.

Among *Bonnaya*, *Lindernia*, *Torenia* and *Vandellia*, *Lindernia* shows seeds without alveolated endosperms and others with alveolated endosperms. *Bonnaya* shows clavate staminodes without appendages, and *Torenia* and *Vandellia* with all stamens perfect. *Torenia* has connate sepals and *Vandellia* has free sepals, joined at base.

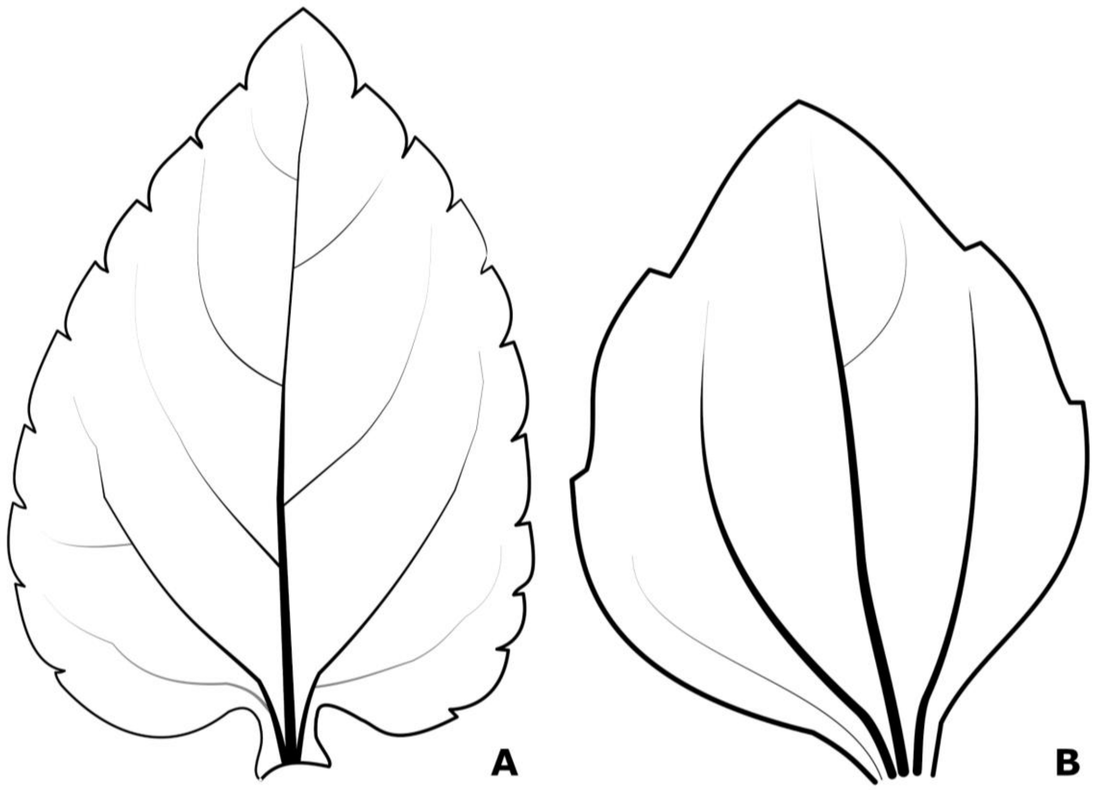

Figure 3. Leaf types, based on venation. A, pinnate leaf (*Torenia anagallis*); B, palmate leaf (*Lindernia rotundifolia*).

Morphological characters are followed briefly.

i. Habit: Except two semi-woody Brazil endemics, *Ameroglossum* and *Stemodiopsis*, Linderniaceae are prostrate to erect herbs. A few prostrate *Torenia* show trailing habit.

ii. Leaves: Leaves are predominantly serrate except for *Lindernia*. Similarly, leaf venation is pinnate in all genera except *Lindernia* (Fig 3).

iii. Inflorescence: Inflorescence varies from true axillary-solitary to pseudo-axillary, solitary to raceme, etc. Solitary flowers mostly arise from the leaf axis; however, sometimes solitary flowers are subtended by bract. Racemes are borne both as axillary and terminal. Less frequently pseudo-umbel inflorescence can be found.

iv. Calyx: Calyx lobes are always free, except connate calyx in *Torenia*.

v. Corolla: Corolla is characterized by two lip, *i.e.*, emarginate to the entire upper lip and three lobed lower corolla lip. Throat of the corolla is mostly protected by the calyx lobes and a small portion of the throat is exposed.

vi. Stamens: Out of two pairs of the stamens, a smaller pair is attached to the upper lip and longer pair to the lower lip. The longed pair is always modified into staminodes or, evolved with swollen geniculation or appendages. Anthers are bi-thecous.

vii. Gynoecium: The bi-lipped, spatulate stigma is attached to the ovary through a long style.

vii. Fruit: Fruits are septicidal to poricidal capsule, with two valves.

ix. Seeds: Seeds are minute and numerous per capsule. Seeds are primarily of two types, aulacospermous and bothrospermous.

1.7. Anatomy

Jung *et al*. (2008) reported the presence intermediate of honeycomb to hollow aerenchyma in the shoots of two emergent aquatic species of *Lindernia*, *L. dubia* and *L. procumbens*. The roots of these two species show radial lysigeny (Jung *et al*. 2008). The

Australian endemic, *Lindernia* subg. Didymadenia is defined by 2-celled blister glands, which are elliptic in outline, found midway between upper and lower surfaces of leaves, and are often evident through cuticle externally (Barker 2018).

1.8. Pollination

Linderniaceae are mostly dependent on the insects for their pollination. Bailey (1882) noted the cross pollination of *Torenia asiatica*. Jin *et al*. (2022) reported the touch sensitive nature of stigma in Linderniaceae, *i.e.* stigma lips close as they come into contact with pollen.

Brazilian endemic genus, *Ameroglossum* Eb.Fisch., S.Vogel & A.V.Lopes, restricted to four states, Rio Grande do Norte, Paraíba, Pernambuco and Alagoas, is known as hummingbird pollinated (Almeida *et al*. 2021; Wanderley 2014). Interestingly, hummingbirds and their morphological diversity have been suggested as the driving force for the speciation of *Ameroglossum*.

1.9. Economic importance

Several species of Linderniaceae are widely used in medicinal, ornamental or traditional purposes. *Torenia fournieri* or Wishbone flower is used extensively as an ornamental plant. The (slightly) sweet flowers of *T. fournieri* are also known to be used in the salads and decoration of dishes. Moisture, protein, ash, and lipid content of the blue flowers are 91.5%, 0.3%, 0.6% and 0.6%, respectively (and 88.9%, 0.5%, 0.7% and 0.7% in violet flowers) (de Morais *et al*. 2020; Mariutti *et al*. 2021).

Some members of Linderniaceae are also used in the treatment of several illness. *Legazpia polygonoides* (Benth.) T. Yamaz. is reported as a remedy in the urinary tract infections, *Lindernia procumbens* (Krock.) Philcox and *Torenia travancorica* Gamble in gonorrhea, *Torenia thouarsii* (Cham. & Schltdl.) Kuntze in for ulcers, *Vandellia anagallis* (Burm. f.) T. Yamaz. (*Gadaga-vel*, in Maharashtra) for sexually transmitted diseases and urethral diseases, etc. (Khare 2009). In the Peninsular Malaysian traditional medicine system, pounded whole plants of *L. polygonoides* are used externally to cure leg sores, ulcers and dropsy. Also, the decoction of whole plants are used in urinary tract problems (Lemmens & Bunyapraphatsara 2003).

Thuan *et al*. (2007) and Satria *et al*. (2017) reported anti-oxidant, antiproliferative activity and strong antioxidant potential activities from *Picria fel-terrae*. Studies also showed that *Torenia concolor* has analgesic, anti-inflammatory, anti-atherosclerotic, and antioxidant activities (Lin *et al*. 2009, Cheng *et al* 2020, Liang *et al*. 2017, Chou *et al.* 2009). Ethanolic extracts of *T. concolor* var. *farmosa* also regulates lipid metabolism (anti-obesity) (Liang *et al*. 2017). Methanolic extracts of *T. concolor* also show cytotoxicity against the NUGC cell line (Ding. *et al.* 2005). Ethanolic extract of *Bonnaya ruelloides* shows anti-HBV activities (Wei *et al.* 2018).

1.10. Chemical constituents

Passon *et al*. (2020) investigated phenolic compounds of three species of Linderniaceae, two desiccant-tolerant, *viz. Craterostigma plantagineum* and *Linderniella brevidens*, and one non-desiccation-tolerant, *Vandellia subracemosa* with ultra-high-performance liquid chromatography electrospray ionisation mass spectrometry (UHPLC-ESI-MS). A total 12 compounds were isolated, among them eight were subclass of phenylethanoid glycosides and one was flavone, luteolin hexoside pentoside. Verbasoside, verbascoside hexoside or caffeoyl verbascoside, verbascoside pentoside, verbascoside, isoverbascoside pentoside, two isomers of verbascoside, and leucosceptoside A, were the identifiable phenylethanoid glycosides. Verbascoside was present in thrice the species. The study suggested the verbascoside might play important roles in resurrection and protective systems of *C. plantagineum* and *L. brevidens*.

Apart from above mentioned physically important chemical constituents, a good number of medicinally important compounds have been isolated from different species of the family. *Picria fel-terrai* is one of the most studied species. Wang *et al*. (2004) isolated six compounds from the ethanolic extract of *P. fel-terrai*, namely, N-benzoylphenylalanyl-L-phenylalaninol acetate, 1-hydroxy-7-hydroxymethyl-9,10-anthraquinone, 9, 16-dioxo-10,12,14-octadeca-trienoic acid, 5,7,4'-trihydroxy-flavone, beta-sitosterol, and daucosterol. Zou *et al*. (2005) isolated three new phenylethanoid glycosides, picfeosides A–C, and five known compounds, *viz.*, wiedemannioside, acteoside, acteoside isomer, cis-acteoside isomer, and cis-acteoside. Thuan *et al*. (2007) reported1-O-3,4-(dihydroxyphenyl)-ethyl-β-D-apiofuranosyl-(1→4)-α-L-rharmnopyranosyl-(1→3)-4-O-caffeoyl-β-D-glucopyranoside from P. fel-terrai.

Ding. *et al.* (2005) reported lupeol, β-sitosterol and stigmasterol, betulin, betulinic acid, oleanolic acid, maslinic acid, alphitolic acid, 3-epimaslinic acid, augustic acid, and β-sitosterol-3-0-β-D glucoside from methanolic extracts of *Torenia concolor*. Chou *et al.* 2009, additionally, reported phenylethyl-O-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside (torenoside A), 20-O-3,4-dihydroxy-β-phenylethoxy-O-α-L-rhamnopyranosyl-(1"→30')-(40'-O-caffeoyl)-β-D-glucopyranoside (torenoside B), acetoside, alphitolic acid, augustic acid, betulin, betulinic acid, (2R,3R)-2,3-bis(3,4-dihydroxy) butyrolactone, campneoside II, 3-epimaslinic acid, isoacetoside, jionoside C, lupeol, maslinic acid , oleanolic acid, succinic acid, stigmasterol and β-sitosterol, β-sitosterol-3-O-D-glucoside from methanolic extracts of *T. concolor*.

Linderruelliosides A, linderruelliosides B, desrhamnosylverbascoside, plantainoside A, and acteoside, were reported by Wei *et al.* 2018, from ethanolic extract of *Bonnaya ruelloides*.

1.11. Knowledge gap

A good number of articles contain mis-identification of Linderniaceae plants, where the events are easily identifiable. In the above-mentioned example of Khisha *et al.* (2012), the ethnobotanical use of *Torenia travancorica* is reported from Bangladesh, a country situated eastward of India, whereas *T. travancorica* is restricted to Southern Peninsular India (Saldanha 1966, Prasad and Sunojkumar 2015). *T. travancorica* is endemic and grows only in the hills of Travancore and Tirunelveli of Southern Western Ghats. Here, the misleading information of the use of *T. travancorica* in medicinal purpose might be fatal.

Another example is the "*Lindernia micrantha* D. Don (Linderniaceae): a red listed plant species new discovery to Tamil Nadu, India", where, Velayudham *et al.* (2020) misidentified the *Bonnaya oppositifolia* as *Vandellia micrantha* (syn. *Lindernia micrantha*). Although the description somewhat matches *V. micrantha*, the photographs of live and herbarium specimens don't. The leaves of the plants in the photographs are serrate (vs entire or undulating in *V. micrantha*), inflorescence raceme (vs axillary solitary), plants densely leaved (vs slender) (Liang and Wang 2014; eFloras 2022).

Secondly, after Grierson and Long (2001), no taxonomic works have been carried out on the Linderniaceae members in the Eastern Himalayan region, despite different changes in the systematic treatments of several genera. Therefore, nomenclatural, as well as, systematic revision is needed for the species restricted to the Eastern Himalayan region.

Therefore, in order to aid in better understanding the taxonomic identity of these species, this dissertation aims to

A. Taxonomic revision of the family Linderniaceae from study area based on comparative morphology.

B. To determine the phenology and phytogeography for each species.

C. To study of detailed Macro and micro-morphological characters of targeted taxa.

D. To explore the Ultra-morphological peculiarities of Pollen grains and seeds.

E. To re-examine the systematic positions of each species.

F. To review the Taxonomic and nomenclatural problems of the studied species.

G. Reconstruction of similarity dendrogram and cluster analysis on the basis of various taxonomic evidences to understand the interrelationship among the members using various bioinformatics tool.

H. To determine the floral morphology and potential pollinators of some selected species.