

Biogeography of *Zehneria* (Cucurbitaceae) and a New Species from India

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Abstract—*Zehneria* is one of the most diverse genera in Cucurbitaceae with 75 accepted species mainly in Southeast Asia and tropical Africa. Here, we describe *Zehneria neorensis*, a new species from Neora Valley in the mountains of West Bengal, India, which has 7–10 cm long twisted fruiting pedicels, the longest pedicels reported in the genus so far. Based on morphological data, we also suggest the transfer of *Melothria morobensis* to the genus *Zehneria*. With a molecular phylogenetic and global biogeographic analysis based on 3856 nucleotides of plastid and nuclear ribosomal DNA, we demonstrate that the genus *Zehneria* most likely originated on the African continent 24 (30–19) million years ago and spread from there at least five times to Madagascar and three times to Asia. *Zehneria neorensis* represents an independent colonization event from Africa to India about 11 (15–7) million years ago. Three lineages reached New Guinea/Australia and finally moved into Polynesia. We infer a rate of at least 20 long-distance dispersal (LDD) events per 10 million years in the genus. This high LDD frequency is most likely a result of the small berry fruits and small flattened seeds of *Zehneria*, which seem perfectly adapted to long-distance bird dispersal. Field observations are needed to investigate a potential effect of the newly discovered extended and coiling pedicels in *Zehneria neorensis* on seed dispersal efficiency. The new species adds to a growing list of rather old Cucurbitaceae lineages in the Himalayan foothills, supporting the hypothesis of long climatic stability in the region.

Keywords—Bird dispersal, coiling fruiting pedicel, long-distance dispersal, new combination, pedicel length.

The genus *Zehneria* Endl. (Cucurbitaceae) is distributed from Africa, Madagascar, and Asia to Australia and the Pacific Islands and currently comprises 75 accepted species (Schaefer 2020). The circumscription of *Zehneria* Endl. has changed considerably over the last centuries and particularly in the last two decades (see Dwivedi et al. 2018 for details) but the consensus of all comprehensive molecular studies (Kocyan et al. 2007; Schaefer and Renner 2011; De Boer et al. 2015; Dwivedi et al. 2018) is that a monophyletic *Zehneria* must include *Anangia* W.J.deWilde & Duyfjes, *Neoachmandra* W.J.deWilde & Duyfjes, and *Pilogyne* Schrad.

Zehneria is one of the cucurbit genera with small-seeded fleshy berries, often ripening bright red or white and thus probably adapted to dispersal by frugivorous birds. This should increase the frequency of long-distance dispersal events and might explain why it is one of the few genera in the family that is native throughout the Old World, from South Africa to Japan, reaching Australia and some of the most isolated islands in the Pacific, like Fiji and Guam.

Field explorations in Africa and Asia combined with molecular analyses resulted in the discovery of several new *Zehneria* species in the past few decades, e.g. *Z. brevirostris* W.J.deWilde & Duyfjes (De Wilde and Duyfjes 2004), *Z. elbertii* W.J.deWilde & Duyfjes (De Wilde and Duyfjes 2006), *Z. longiflora* G.W.Hu & Q.F.Wang (Wei et al. 2017), *Z. palmatiloba* O.Lachenaud & H.Schaefer. (Lachenaud and Schaefer 2021), and *Z. pedicellata* W.J.de Wilde & Duyfjes (De Wilde and Duyfjes 2006). In 2018, the first authors discovered a *Zehneria* with very long and twisted fruiting pedicels (Fig. 1) during botanical explorations in the Neora Valley National Park in the mountains of West Bengal, India, bordering Sikkim and Bhutan (Fig. 2). Subsequent comparisons with herbarium material and literature research revealed that the plant clearly differs from the six species currently known from India (De Wilde and Duyfjes 2006; Renner and Pandey 2013;

Dwivedi et al. 2018) and all the species reported from Bhutan and China (Grierson and Long 2001; Lu et al. 2009).

Here, we perform a comparison of pedicel length in the genus based on literature data and herbarium specimens combined with a molecular phylogenetic analysis based on DNA sequence data for three plastid regions and the nuclear ribosomal ITS region of 53 *Zehneria* species to test the hypothesis that the plant from Neora valley represents a new species of that genus. Furthermore, we present a comprehensive biogeographic analysis of *Zehneria* to elucidate the origin and age of the Neora valley taxon. This enables us to also test more generally the hypothesis of frequent long-distance dispersal in *Zehneria*, which would support the idea of frugivorous birds as key dispersal agents in the clade.

MATERIALS AND METHODS

Morphology—Specimens were borrowed from or studied during personal visits at the herbaria B, CAL, M, P, and TUM (acronyms according to Index Herbariorum, <http://sweetgum.nybg.org/science/ih/>). Additional material was studied online via JSTOR (<https://plants.jstor.org>). All measurements were conducted on herbarium specimens. We also consulted recent revisions (De Wilde and Duyfjes 2006; Lu et al. 2009) to obtain information on fruiting pedicel lengths.

Taxon Sampling and Sequencing—Material of the Neora valley plant was sent to Germany for DNA extraction and sequencing with permission of the National Biodiversity Authority, Chennai, India (NBA/TechApp/9/Form B-171/20/20–21/2602, dt. 28.10.2020). Additional extractions were conducted from old herbarium material received from P. Material from B was selected by the senior author and DNA extraction conducted at Botanical Garden and Botanical Museum Berlin. In total, we obtained material for 60 taxa, all with appropriate permits, covering 53 of the 75 currently accepted species. All the missing species, mainly from New Guinea and the Philippines, have been examined based on digital specimen images on JSTOR (<https://plants.jstor.org>).

Total genomic DNA was isolated from dry leaf material with a commercial plant DNA extraction kit (NucleoSpin, Macherey-Nagel, Düren, Germany), following the manufacturer's manual. For PCR amplification of four chloroplast loci (*trnL* intron, *trnL-trnF* spacer, *rpl20-rps12* spacer,



FIG. 1. *Zehneria neorensis* sp. nov. (a) Habit. (b) Leaf surface. (c) Lower side of leaf. (d) Male inflorescence. (e) Male flower. (f) Female flower. (g) Long-pedicellate fruit. (h) Fruit detail. (i) Immature seeds.



FIG. 2. Maps of (a) India, (b) West Bengal, and (c) Neora Valley National Park with locations of the *Zelmeria neorensis* populations (green dots). Background maps by Esri, DigitalGlobe, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AeroGRID, IGN, and the GIS User Community.

and *matK* gene) and the nuclear ribosomal ITS1 + 5.8S+ITS2 region, we used the KAPA2G Fast HotStart Ready Mix (KAPA Biosystems, Wilmington, USA) and the manufacturer's protocol. For amplification and sequencing, primers for the ITS1 + 5.8S+ITS2 regions were described by Balthazar et al. (2000), for the *trnL* intron and *trnL-trnF* spacer by Taberlet et al. (1991), for *rpl20-rps12* by Hamilton (1999), and for *matK*, the primers were AF and 8R and internal primers F1 and R1 of Ooi et al. (1995). The PCR amplicons were purified using exonuclease I and shrimp alkaline phosphatase following the manufacturer's protocol (Jena Bioscience, Jena, Germany). Sequencing reactions consisted of 2.5 μ L clean PCR product, 5 μ L H₂O, and 2.5 μ L primer (10 μ M). Fourteen sequences were newly generated for this study, and combined with our available *Zehneria* datasets (Dwivedi et al. 2018; Lachenaud and Schaefer 2021). Genbank accession numbers and vouchers are listed in Appendix 1.

Sequence Alignment and Phylogenetic Analyses—Sequences were edited and aligned with Geneious v. 11 (Biomatters Ltd., Auckland, New Zealand). The aligned plastid matrix comprises 2945 nucleotides and the ITS matrix 911 nucleotides.

Phylogenetic analyses were performed using RAxML v. 8.1.18 (Stamatakis et al. 2008; Stamatakis 2014) and MrBayes v. 3.3.6-svn(r1040) x64 (Ronquist and Huelsenbeck 2003) on the CIPRES Science Gateway v. 3.3 (Miller et al. 2010). RAxML searches relied on the GTR + G + I model, which was the best fit according to jModelTest2 (Darriba et al. 2012). For the MrBayes analysis, we executed six runs in parallel with four chains each running for 50 million generations and sampling every 1000 generations. We used Tracer v. 1.6.0 (Rambaut et al. 2014) to check for convergence of the chains. Specifically, we visually inspected the Tracer plots and further checked that all ESS values were above 200. After exclusion of the first 20% of the sampled trees, we computed a Bayesian majority-rule consensus tree of the remaining 240,000 trees with the *alcompact* command. Each DNA region was analyzed separately first, then with all plastid regions concatenated. Finally, in the absence of well-supported conflict, the nuclear and plastid datasets were concatenated and analyzed together. All trees were rooted on *Cucurbita pepo* L. as most distant outgroup and a group of more closely related relatives representing most genera of the tribe Benincaseae.

Molecular Clock and Biogeographic Analyses—Bayesian molecular clock estimation and inference of ancestral ranges was performed in BEAST v. 1.8.4 (Drummond and Rambaut 2007) on the CIPRES Science Gateway (Miller et al. 2010) using the combined plastid plus nuclear dataset. The molecular clock estimation was performed under a log normal

relaxed clock uncorrelated-rates model and run with a birth-death tree prior and a GTR + G + I model with six gamma categories. In the absence of any *Zehneria* fossil records, we chose a secondary calibration approach and calibrated our analysis with four secondary calibration points from an earlier Cucurbitaceae-wide analysis that had used four fossil and geological calibration points (Schaefer et al. 2009) and a recent phylotranscriptomic study (Guo et al. 2020). The following constraints were used, each with a normal prior distribution: (1) root age (split *Cucurbita pepo* vs. rest) set to 39 (32.2–48.2) Mio years; (2) split *Melothria-Indomelothria* set to 15 Mio years, SD 4; (3) split *Cucumis-Muellerargia* set to 16 Mio years, SD 3; (4) split *Coccinia-Diplocyclos* set to 15 Mio years, SD 3; (5) split *Lagenaria-Peponium* set to 12 Mio years, SD 3.

To infer ancestral ranges, the distribution of extant *Zehneria* species and the outgroups was classified into six different categories (Africa, Americas, Australia and Polynesia, India, Madagascar, and Southeast Asia) and analyzed under a symmetric continuous time Markov chain (CTMC) model for discrete state reconstructions as implemented in BEAST v. 1.8.4 (Drummond and Rambaut 2007).

We ran four Markov chain Monte Carlo (MCMC) chains for 50 million generations, sampling every 1000th generation to infer the phylogeny, divergence time and ancestral ranges in the same analysis. Of the 50,001 posterior trees, we discarded the first 5000 trees as burn-in. Convergence of the MCMC chains was checked with Tracer v. 1.6.0 by inspection of the plots and the effective sample size (ESS) values (all > 200). The trees were summarized using TreeAnnotator v. 1.8.0. In total, 45,001 trees were combined into the final chronogram, which was visualized in FigTree v. 1.4.2 (Rambaut 2009).

RESULTS

Morphology—The comparison of the list of accepted *Zehneria* species with our sampling revealed 22 missing species (Table 1), but none of them has a fruiting pedicel length similar to the Neora valley plants. For the few species where pedicel length is unknown, leaf shape did not match our plant. We included *Melothria morobensis* Merr. & L.M.Perry (Merrill and Perry 1948) from New Guinea in the morphological comparison because the species had already been transferred to

TABLE 1. Species not included in the molecular analyses, with geographic origin and pedicel length.

Taxon	Geographic origin	Fruiting pedicel length (in mm)
<i>Z. angolensis</i> Hook.f.	Angola	1–3
<i>Z. backeri</i> (W.J.deWilde & Duyfjes) H.Schaefer. & S.S.Renner	Java, Lesser Sunda Islands	20–45
<i>Z. breviostris</i> W.J.deWilde & Duyfjes	Thailand	8–12
<i>Z. clemensiae</i> (Merr. & L.M.Perry) M.Dwivedi, A.K.Pandey & H.Schaefer.	New Guinea	30–45 in flower, fruits not known
<i>Z. idenburgensis</i> (Merr. & L.M.Perry) M.Dwivedi, A.K.Pandey & H.Schaefer.	New Guinea	10
<i>Z. immarginata</i> W.J.deWilde & Duyfjes	Lesser Sunda Islands (Lombok, Flores)	15–25
<i>Z. lancifolia</i> (W.J.deWilde & Duyfjes) H.Schaefer. & S.S.Renner	New Guinea	unknown
<i>Z. macrantha</i> (W.J.deWilde & Duyfjes) H.Schaefer. & S.S.Renner	Philippines (Luzon)	unknown
<i>Z. madagascariensis</i> Keraudren	Madagascar	10
<i>Z. martinez-crovettoi</i> Keraudren	Madagascar	6–10 in flower, fruits not known
<i>Z. morobensis</i> (Merr. & L.M. Perry) H.Schaefer. comb. nov. basionym: <i>Melothria morobensis</i> Merr. & L.M. Perry, J. Arnold Arbor. 29: 167. 1948.	New Guinea	15
<i>Z. pedicellata</i> W.J.deWilde & Duyfjes	New Guinea	15–25
<i>Z. platysperma</i> (W.J.deWilde & Duyfjes) H.Schaefer. & S.S.Renner	Thailand	5–7
<i>Z. repanda</i> (Blume) C.M.Simmons	Indonesia, Philippines, Taiwan	3–6
<i>Z. ridens</i> Verdc.	Tanzania	3–3.5
<i>Z. rizalensis</i> (W.J.deWilde & Duyfjes) Pelsler & H.Schaefer.	Philippines	2–4
<i>Z. scaberrima</i> (Merr.) Pelsler & H.Schaefer.	Philippines	20–45
<i>Z. somalensis</i> Thulin	Somalia	25–40
<i>Z. subcoriacea</i> Y.D.Zhou & Q.F.Wang	East Africa	unknown
<i>Z. tenuispica</i> W.J.deWilde & Duyfjes	India (Assam), Thailand, Myanmar	min. 25
<i>Z. trichocarpa</i> W.J.deWilde & Duyfjes	Philippines (Nutol)	3–5
<i>Z. trullifolia</i> W.J.deWilde & Duyfjes	Celebes	unknown

the genus *Neoachmandra* by De Wilde and Duyfjes (2006) based on a detailed morphological analysis. We could not produce sequence data for this species but based on leaf and flower morphology and our earlier findings that *Neoachmandra* is nested in *Zehmeria* (Dwivedi et al. 2018), this taxon is here transferred to the genus *Zehmeria* (Table 1).

Molecular Data—In the sequence for the ITS1–5.8s–ITS2 region, *Z. neorensis* has 8 autapomorphic sites, causing a relatively long branch in the phylogeny estimate. Its position in the ITS phylogeny (Supplemental Fig. S1, Ranjan et al. 2022) is in a polytomy formed by the *Zehmeria* s.s. clade (with RAxML bootstrap support (BS) = 71), the *Pilogyne* clade



FIG. 3. Phylogeny estimate based on 3856 nucleotides of chloroplast and nuclear ribosomal DNA, inferred with RAxML (Stamatakis 2014). RAxML bootstrap (BS) values ≥ 60 and Bayesian posterior probability (BPP) ≥ 0.85 from an independent MrBayes run (Ronquist and Huelsenbeck 2003) are indicated at the nodes. The newly described *Zehmeria neorensis* is highlighted in red, the remaining Indian *Zehmeria* species in blue letters. The BS and BPP values supporting the phylogenetic distinctness of the new species are also highlighted in red.

(BS = 90) and *Z. neorensis*, *Z. minutiflora*, *Z. oligosperma*, *Z. pallidinervia* and *Z. peneyana*, as well as *Z. anomala* on isolated branches. There is low support (BS = 61) for grouping *Z. neorensis* and *Z. anomala* with the *Pilogyne* clade.

In the plastid sequence data, *Z. neorensis* shows 35 autapomorphic sites, resulting again in a rather long branch in the phylogeny estimate (Supplemental Fig. S2, Ranjan et al. 2022). The plastid phylogeny shows again the two main clades (*Zehneria* s.s. with BS = 98 and *Pilogyne* with BS = 82 and *Z. anomala*, *Z. minutiflora*, and *Z. pallidinervia* unplaced. Both, *Zehneria neorensis* and *Z. oligosperma* are grouped in the *Pilogyne* clade but without well-supported sister taxa. We failed to produce plastid sequences for *Zehneria peneyana*.

The combined plastid and nuclear alignments produced nearly identical phylogeny estimates in the MrBayes and RAxML analyses. The only difference is found outside the *Zehneria* clade, where the Bayesian analysis placed *Benincasa* and *Citrullus* as sister to all other Benincaseae, while the ML analysis groups *Benincasa* with the clade consisting of *Blastania*, *Dactyliandra*, and *Trochomeria*. We therefore chose to show in Fig. 3 the ML tree with both BS and Bayesian posterior probability values (BPP) from the MrBayes analysis plotted on the branches. In this combined evidence tree, *Z. anomala* and *Z. minutiflora* are subsequent sisters to all other species with good support (Fig. 3). *Zehneria oligosperma* groups with the *Zehneria* s.s. clade with weak bootstrap (BS = 73) but full BPP support (1.0), while *Z. neorensis* is well-supported as part of the *Pilogyne* clade (BS = 85, BPP = 1.0). Within the *Pilogyne* clade, *Z. neorensis* is sister to the remaining species and while the BS value for the sister relationship is very low, there is better support (BPP = 0.95) from the Bayesian analysis (Fig. 3; Supplemental Fig. S3, Ranjan et al. 2022). Besides this, its long branch clearly indicates its distinct genetic identity far from any other Indian taxa (highlighted in blue in Fig. 3).

We therefore describe the Neora valley plant in the following as a new species of *Zehneria* most likely endemic to West Bengal, India.

TAXONOMIC TREATMENT

Zehneria neorensis Ranjan, Anant Kumar, G. Krishna & H. Schaefer. sp. nov. TYPE: INDIA. West Bengal: Kalimpong district, Neora Valley National Park, Lava to Kolakham,

27.091776N N, 88.681313 E, 1798 m, 29 Sep 2018, V. Ranjan, G. Krishna & A. Kumar 77931 (holotype: CAL!; isotype: TUM!); Ambik to Panchpokhri, 27.025654N, 88.71078 E, 1037 m, 02 Oct 2018, V. Ranjan, G. Krishna & A. Kumar 77979; Mouchuki, 27.028865N, 88.781648E, 1290 m, 23 Sep 2019, V. Ranjan, G. Krishna & A. Kumar 86103 (paratype: CAL!, isoparatypes: CUH!). Figure 1

The new species can be distinguished from all other known *Zehneria* species in the length of the fruiting pedicels (7–10 cm) and its coiling appearance. It differs from *Z. marlothii* (Cogn.) R.Fern. & A.Fern. (close in chloroplast phylogeny) and *Z. bodinieri* (H.Lév.) W.J.deWilde & Duyfjes (close in geographical range and overall morphology) by the characters indicated in Table 2.

Scandent herb, monoecious. Stems 2–3 mm in diam; branches slender, sulcate-angular, glabrous or sparsely pubescent; tendrils filiform, simple, up to 15 cm or more long, glabrous. Leaves simple, triangular-ovate to broadly triangular-ovate in outline, 2–8.5 × 1.5–8 cm, truncate at base, sinuate-toothed to entire (apical region) at margins, rather shallowly to moderately deeply palmately 5–7-lobed, acuminate to apiculate at apex, thinly coriaceous, green shiny and scabrid on upper surface, glabrous beneath except veins; 5–7-palmately nerved at base, veins hairy on both surfaces; petioles slender, 1–4 cm long, sparsely hairy. Male inflorescences axillary, pedunculate, up to 15-flowered, racemiform, often with solitary, coaxillary, pedicellate flower; peduncles 3–5 cm long, green, grooved, glabrous; rachis 5–9 mm long, glabrous; pedicels 1–6 mm long. Calyx campanulate, green; tube ca. 1 mm, glabrous; teeth 5, minute. Corolla campanulate, white; tube ca. 1 mm, densely hairy inside, glabrous outside; lobes 5, triangular, ca. 1 mm long, spreading at anthesis, acute at apex, hairy. Stamens 3, inserted at the bottom of corolla tube; filaments distinct, linear, ca. 1 mm long, glabrous; anthers yellowish, basifixed, thecae 2; connective produced; disk 3-lobed, ca. 1.5 mm in diam. Female inflorescences axillary, solitary or pedunculate racemiform, 2–3-flowered; peduncles 1.5–1.8 cm long; rachis ca. 1 cm long; pedicels ca. 2 mm long, accrescent. Calyx and corolla same as in male flowers. Ovary globose with ca. 0.5 mm long neck; style very short; stigma discoid, indistinctly 3-lobed; disk prominently 3-lobed. Fruits green to pale green, baccate, globose, 1–1.4 cm in diam, tipped at apex, smooth, glabrous; fruiting pedicels filiform, 7–10 cm long, coiling, glabrous. Seeds (2)–3, ovate-oblong, 6–8 × 4–5 mm, compressed, smooth, marginate.

TABLE 2. Distinguishing characters between *Z. neorensis* sp. nov., *Z. marlothii*, and *Z. bodinieri*, key characters highlighted in bold letters.

Characters	<i>Z. neorensis</i> sp. nov.	<i>Z. marlothii</i>	<i>Z. bodinieri</i>
Leaves	Triangular-ovate to broadly triangular-ovate in outline, 2–8.5 × 1.5–8 cm, truncate at base , margins shallowly to moderately palmately 5–7-lobed	Ovate or broadly ovate in outline, 1.3–7.0 × 1.0–7.8 cm, cordate at base, margins shallowly to moderately palmately 3–5-lobed	Broadly ovate or rarely triangular-ovate, 4–12 × 3–10 cm, base semicircular, rarely subtruncate, margins undulate-denticulate, undivided or sometimes shortly 3–5-lobed
Male inflorescences	Pedunculate, up to 15-flowered, racemiform, often with solitary coaxillary flower with long pedicel; peduncles 3–5 cm long, grooved, glabrous	Pedunculate, 2–15 flowered in shortly racemiform or subumbelliform axillary clusters, rarely solitary; peduncles 0.2–2 cm long	Pedunculate, 3–9-flowered, subcapitate or cymose; peduncles 1–4 cm long, slender, glabrous
Fruits	Fruits green to pale green , globose, 10–14 mm in diam; fruiting pedicels filiform, 7–10 cm long, coiling	Fruits red, globose, 5–7 mm in diam; fruiting pedicels 2–7 mm long, not coiling	Fruit red when ripe, globose, 10–14 mm in diam; fruiting pedicels 3–10 mm long, not coiling
Seeds	Seeds ovate-oblong, 6–8 × 4–5 mm , compressed, smooth, marginate	Seeds ovate, 4.2–4.7 × 2.7–3.5 mm, compressed, smooth	Seeds ovate-oblong, ca. 5 × 3–3.5 mm, compressed, smooth, marginate

Phenology—Flowering: August–October; fruiting: September–November.

Etymology—The specific epithet refers to the type locality in the Neora river valley. The Neora river is the prime source of water for the area and gave the name to the Neora Valley National Park, established in 1986, which is an important site for conservation of the endangered Red Panda (*Ailurus fulgens*, Mammalia).

Distribution and Habitat—The population of *Zehneria neorensis* covers four forest areas: the Kolakham forest block with 5–6 individuals, Amblok forest block with 3–4

individuals, Dolay forest block with 30–35 individuals, and Mouchuki forest block with 6–7 individuals, all located in Neora Valley National Park, Kalimpong District, West Bengal, India. The species was found at an elevational range of 1000–1800 m a.s.l. and grows on outcrops of shaded moist hillslopes in subtropical evergreen forest with *Hydrangea febrifuga* (Lour.) Y.De Smet & Granados, *Maesa chisia* D.Don, *Girardinia diversifolia* (Link) Friis, *Globba multiflora* Wall. ex Baker, *Impatiens spirifera* Hook.f. & Thomson, and *Strobilanthes multidens* C.B.Clarke (Fig. 2).

KEY TO THE SPECIES OF ZEHNERIA IN INDIA BASED ON FLOWERING AND FRUITING MATERIAL

1. Plants dioecious *Z. hookeriana* (Wight & Arn.) Arn.
1. Plants monoecious 2
 2. Fruiting pedicels 7–10 cm long, twisted *Z. neorensis* Ranjan, Anant Kumar, G. Krishna & H. Schaefer. sp. nov.
 2. Fruiting pedicels < 7 cm long, not twisted 3
 3. Fruits fusiform *Z. thwaitesii* (Schweinf.) C. Jeffrey
 3. Fruits globose or oblong, ellipsoid but not fusiform 4
 4. Ovary and fruits ellipsoid *Z. maysorensis* (Wight & Arn.) Arn.
 4. Ovary and fruits globose 5
 5. Male flowers solitary or 2 or 3 in a raceme *Z. japonica* (Thunb.) H.Y.Liu
 5. Male inflorescences with ≥ 3 flowers, spike-like, subcapitate or cymose 6
 6. Male inflorescences 3–9-flowered, subcapitate or cymose *Z. bodinieri* (H.Lév.) W.J.deWilde & Duyfjes
 6. Male inflorescence a 5–15-flowered spike-like raceme *Z. tenuispica* W.J.deWilde & Duyfjes

Historical Biogeography of *Zehneria*—The molecular clock and ancestral range analyses of the combined nuclear and plastid data reveal that the genus *Zehneria* evolved most likely on the African continent between 23 (30–19, stem age) and 14 (23–13, crown age) million years ago (Fig. 4; Fig. S4, Ranjan et al. 2022). From there, the ancestors of today's *Zehneria* species spread at least five times to Madagascar and three times to Asia. From Asia, the genus reached New Guinea and Australia twice and continued twice to Polynesia. *Zehneria neorensis* represents an independent colonization event from Africa to India about 11 (15–7) million years ago, while all the remaining Indian *Zehneria* lineages are much younger (Fig. 4).

DISCUSSION

Our analyses clearly show that the plant from Neora Valley National Park represents a new species of *Zehneria* based on molecular as well as morphological data. The discovery of a new species of that genus in India is not really a surprise, since the diversity of Cucurbitaceae and especially of *Zehneria* in India was found to be unexpectedly low when compared to Southeast Asia or East Africa (Dwivedi et al. 2018), indicating a Linnean shortfall (Brown and Lomolino 1998) or in other words, potential for overlooked species in the region. Furthermore, fieldwork of the senior author in the Himalayan biodiversity hotspot region had already revealed several so far undescribed taxa that probably deserve to be described as new species as well (Dwivedi et al. 2018). *Zehneria neorensis* is, however, very special for two reasons: 1) as we show with our molecular dating analysis, it is 2–3 times as old as all other Indian *Zehneria* species and indeed one of the oldest lineages in the whole genus (Fig. 4); and 2) its morphology, the very long coiling pedicel, is rare in the family and throughout the flowering plants.

With respect to the inferred age: only very few East African savanna species (*Z. anomala*, *Z. minutiflora*, *Z. oligosperma*) are

older than *Z. neorensis* but live on a different continent in very different habitats. Interestingly, the Himalayan foothills, where *Z. neorensis* was discovered, are home to some of the oldest lineages of Cucurbitaceae, e.g. *Indofevillea* Chatterjee, *Zanonia* L., *Schizopepon* Maxim., and *Gynostemma* Blume (Schaefer et al. 2009), which suggests the availability of suitable habitats in the region for a period of at least 40 million years and fits well with the geological scenarios for the Himalayan origin, which infer an uplift of the Tibetan plateau 45–40 million years ago and onset of the monsoon system 35 to 20 million years ago (Favre et al. 2015).

Regarding the morphology, the extremely long and coiling fruiting pedicel of *Z. neorensis* in combination with a green ripe fruit is very unusual. It is by far the longest pedicel known in the genus (Table 1; pedicel length unknown in four species). At the family level, there are a few species with even longer pedicels but they show a very different dispersal biology: all geocarpic species of Cucurbitaceae (*Cucumis humifructus* Stent, *Cucumis umbellatus* I.Telford, *Echinopepon arachnoideus* (Dieterle) A.Monro & P.J.Stafford, and *Kedrostis psammophila* Bruyns) develop several cm or even dm long fruiting pedicels, which allow the fruit to develop underground. This is clearly not the case in *Z. neorensis* which produces small berries hanging on their long pedicels high up in bushes (Fig. 1A). The genus *Schizopepon* also shows very long and sometimes coiling pedicels but as the name indicates, *Schizopepon* fruits split when ripe and release the seeds. This is different from the situation in *Z. neorensis* where frugivorous birds might play a role as dispersal agents. The pale green color of the fruit is, however, unusual for plants adapted to bird-dispersal. It has been suggested that fruits ripening green allow for additional photosynthetic activity and thus the production of more energy-rich fruits (Cipollini and Levey 1991). In *Z. neorensis*, however, the fruits do not seem to be larger or more numerous than in most other *Zehneria* species and therefore, it seems more likely that a novel, unexpected seed dispersal mechanism has evolved. Further fieldwork in Neora Valley, especially observation of feeding

behavior of birds and mammals in the region, might help to answer this question.

More generally, our study reveals an extraordinarily high number of long distance dispersal (LDD) events in *Zehneria*. With up to 20 LDDs inferred in ca. 10 million years, especially in the Pacific (Fig. 4), the rate of 2 LDDs per million years is much higher than the previously inferred rate of 7 LDDs per 10 million years in Cucurbitaceae (Schaefer et al. 2009). While our much more comprehensive sampling obviously allows for a much more precise inference of the biogeographic history than in the family-wide study of Schaefer et al. (2009), the real reason for the high dispersal capability of *Zehneria* is very likely the small berry fruit with small flattened but hard-coated seeds, which seems very well adapted to endozoochorous bird dispersal. Similar fleshy fruits evolved independently in the distantly related cucurbit genera *Bryonia* and *Austrobryonia*, where LDD processes from the Mediterranean throughout temperate Asia and into Australia have been inferred (Schaefer et al. 2008) and in some clades of the melon genus *Cucumis* L. with a significant number of island species (Sebastian et al. 2010; Endl et al. 2018). Epizoochorous bird dispersal across the Pacific has been suggested for the spiny-fruited cucurbit genus *Sicyos*, which reached Galapagos, Hawaii, Australia, and New Zealand from continental South American source regions (Sebastian et al. 2012). Unfortunately, like in most Cucurbitaceae, there is hardly any observational data of fruit and seed dispersal in *Zehneria* available, clearly a research gap that should be addressed with more fieldwork and modern wildlife and surveillance cameras.

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AUTHOR CONTRIBUTIONS

VR, GK, and AK collected the plant material, took photographs, carried out the morphological study, drafted the morphological results, and prepared the photo plate. HS performed the molecular and biogeographical analyses and wrote the final draft; all authors contributed to and accepted the final manuscript.

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- APPENDIX 1. Taxa, Genbank accession numbers (ITS, *matK*, *trnL*, *trnL-F*, *rpl20-rps12*; dash (—) = sequence not obtained), and specimen voucher information of accessions from which DNA was extracted for sequencing.
- Melothria* (*Zehneria*) *tomentosa* Cogn.: MZ409455, —, —, —, —, Ethiopia, Gillett 14688 (B); *Zehneria keayana* R.Fern & A.Fern.: MZ409449, —, MZ409518, MZ409518, MZ409515, Cameroon, De Wilde 7722 (B); *Zehneria monocarpa* G.W.Hu, Ngumbau & Q.F.Wang: MZ409450, —, —, —, —, Tanzania, Usambara Mountains, Peter 56808 (B); *Zehneria neorensis* sp. nov.: MZ409451, MZ398019, MZ409519, MZ409519, MZ409516, India, West Bengal, Neora Valley Ranjan et al. 77931 (TUM); *Zehneria oligosperma* C.Jeffrey: MZ409452, —, MZ409520, MZ409520, MZ409517, Tanzania, Polhill & Paulo 1148 (B); *Zehneria peneyana* (Naudin) Schweinf. & Asch.: MZ409453, —, —, —, —, Madagascar, Toliara, Mahabo, Dequaire 27166 (P); *Zehneria rutenbergiana* (Cogn.) Keraudren: MZ409454, —, —, —, —, Madagascar, Manaka-Est, Rakotovo 11821 (P).