

Supplementary material

**Genome differentiation, natural hybridisation and taxonomic relationships among *Eleocharis viridans*, *E. niederleinii* and *E. ramboana* (Cyperaceae)**

Carlos Roberto Maximiano da Silva<sup>A</sup>, Thaíssa Boldieri de Souza<sup>A</sup>, Rafael Trevisan<sup>B</sup>,  
María Socorro González-Elizondo<sup>C</sup>, José Marcelo Domingues Torezan<sup>D</sup>,  
Rogério Fernandes de Souza<sup>A</sup> and André Luís Laforga Vanzela<sup>A,E</sup>

<sup>A</sup>Laboratory of Cytogenetics and Plant Diversity, Department of General Biology,  
Center for Biological Sciences, State University of Londrina, Londrina, 86051-970, PR, Brazil.

<sup>B</sup>Department of Botany, Federal University of Santa Catarina,  
Florianópolis, 88010-970, SC,  
Brazil.

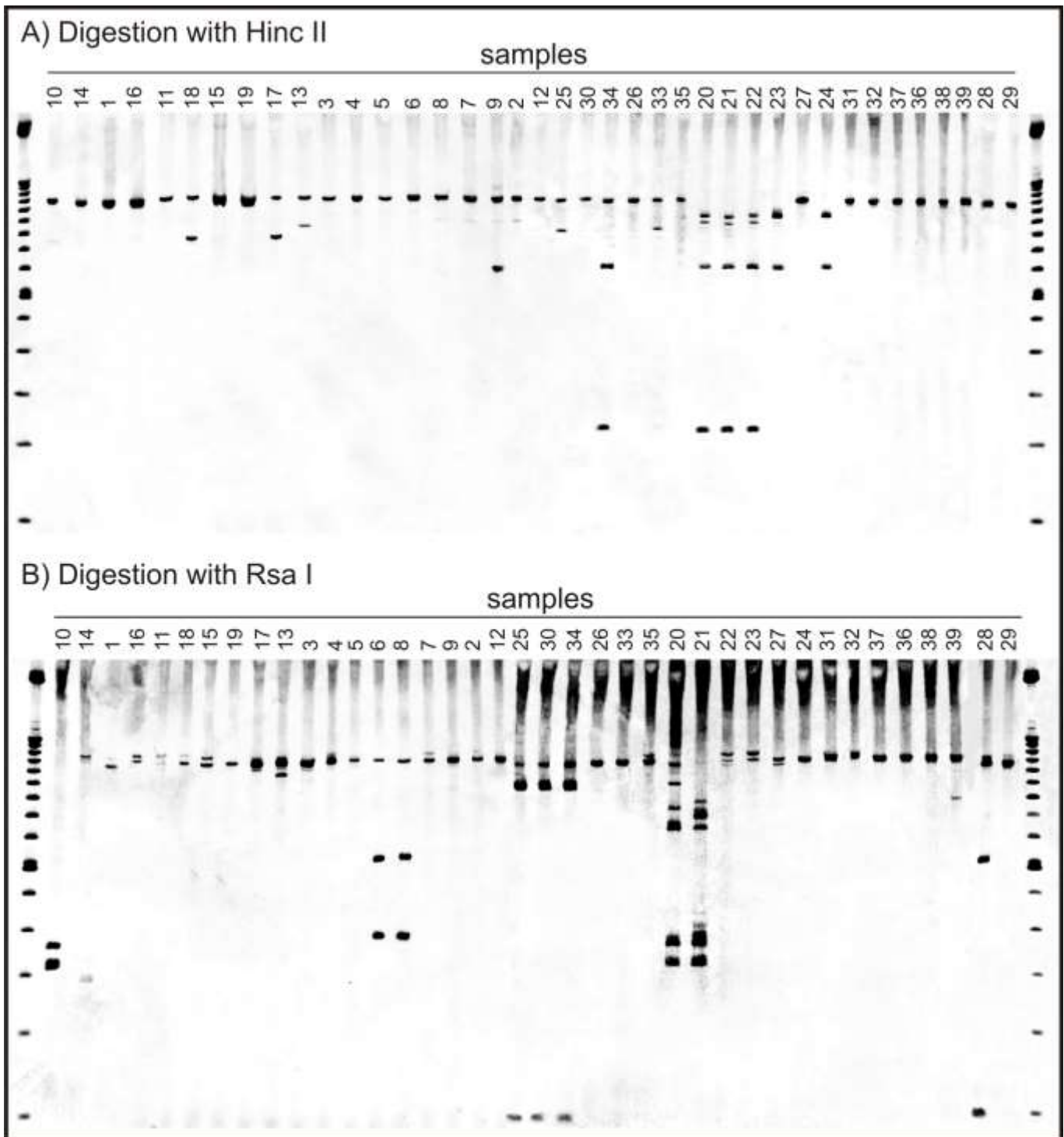
<sup>C</sup>CIIDIR, Instituto Politécnico Nacional, Sigma Fraccionamiento 20 de Noviembre II,  
34220 Durango, Durango, Mexico

<sup>D</sup>Laboratory of Biodiversity and Ecosystem Restoration, State University of Londrina,  
86051-970, PR, Brazil

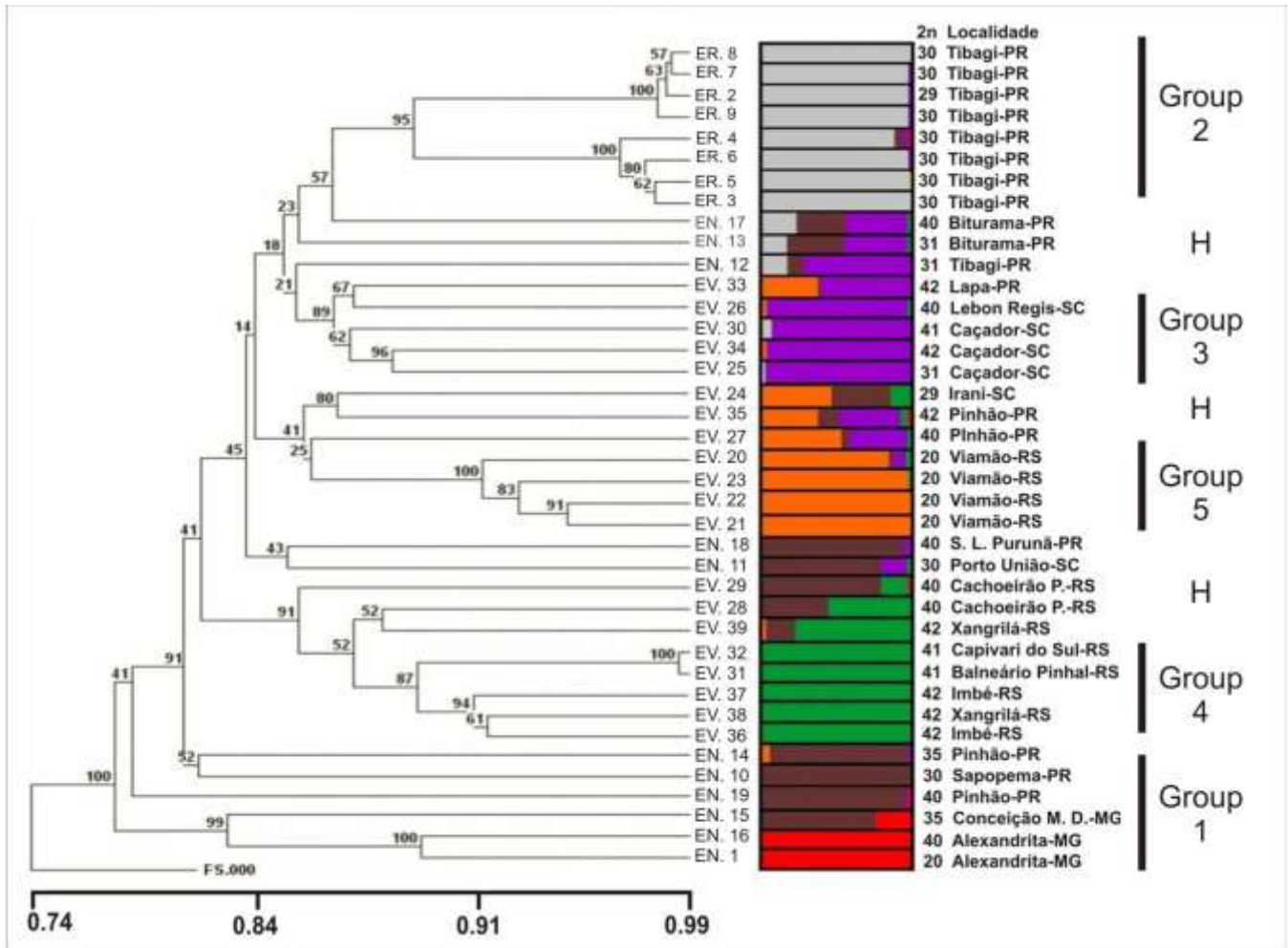
<sup>E</sup>Corresponding author. Email: andrevanzela@uel.br



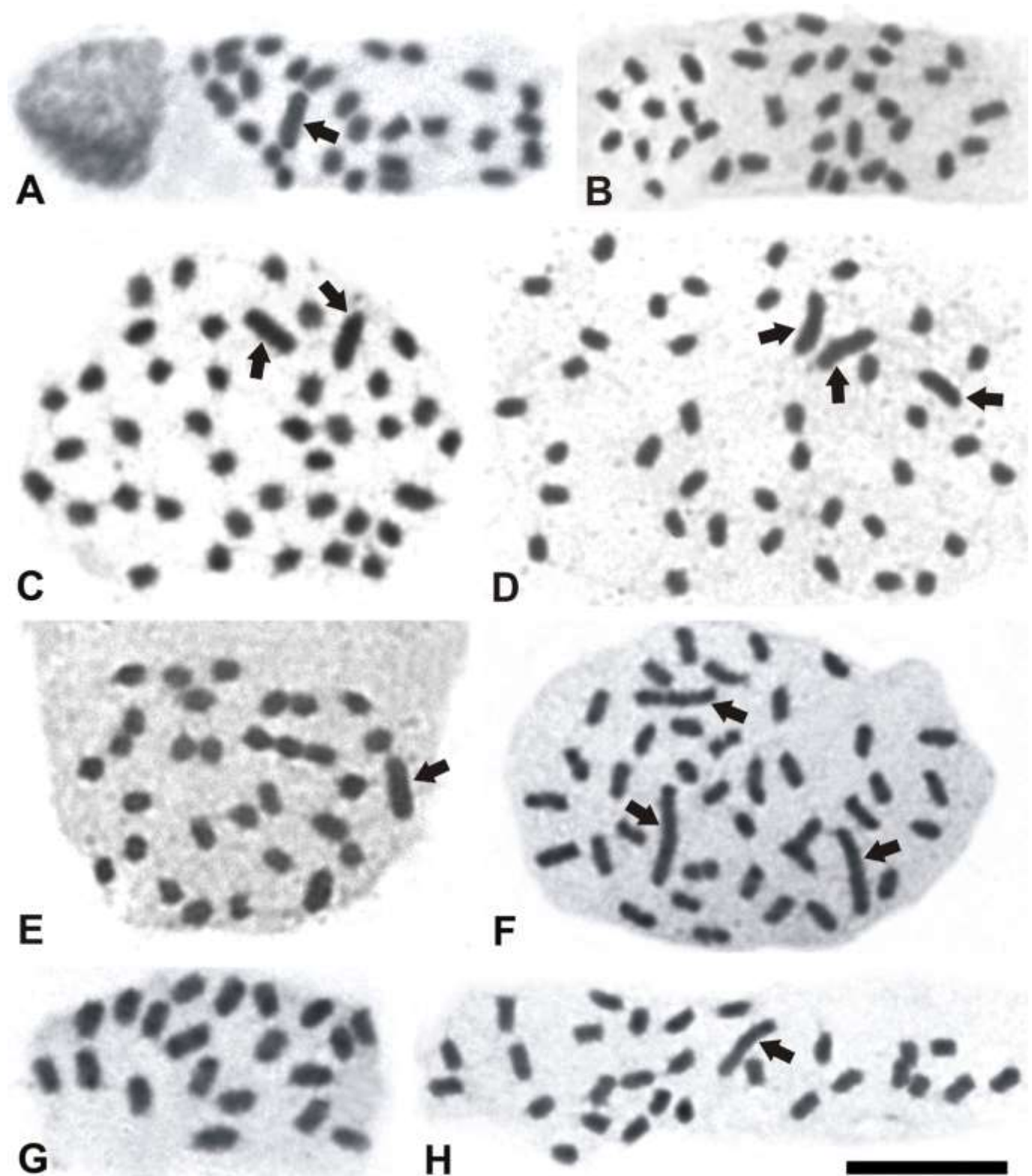
**Fig. S1.** *Eleocharis niederleinii*, showing proliferation in the apex of the culm. Note the ruler for size comparison.



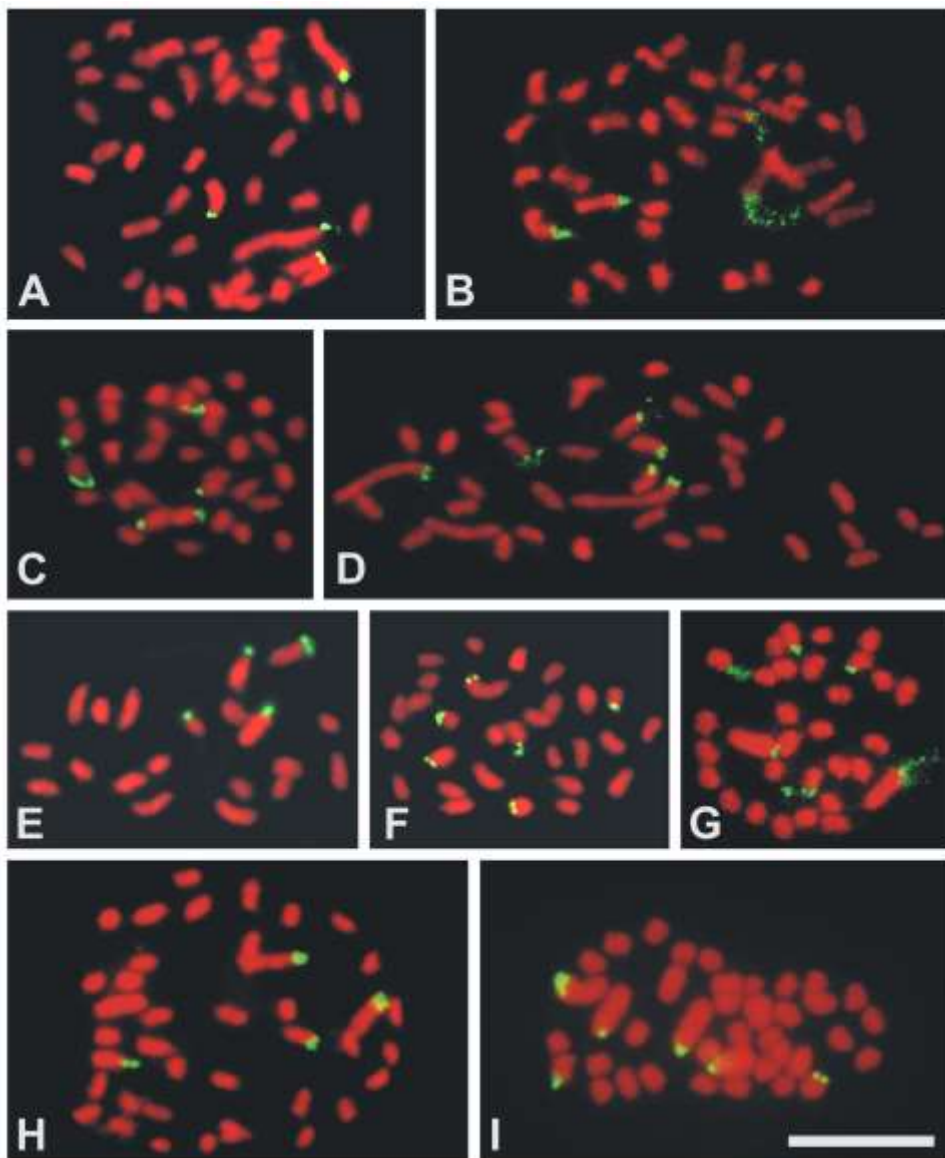
**Fig. S2.** Polyacrylamide electrophoresis. A. Bands of internal transcribed spacer–cleaved amplified polymorphic sequence (ITS-CAPS) digested with *HincII* enzyme. B. Bands of ITS-CAPS digested with *RsaI* enzyme. Numbers represent samples according to Table 1 and Fig. 1A.



**Fig. S3.** Genetic similarity tree of samples generated after random amplified polymorphic DNA (RAPD) analysis, with numbers indicating bootstraps and, below the bar, representing the similarity index, obtained by the method of Nei and Li (1979). The number of each population is given before the colour chart. Chromosome number and the city and province (city-province) of occurrence are indicated after the chart. Mixed colours indicate possible hybrids. EN, *Eleocharis niederleinii*; ER, *E. ramboana*; and EV, *E. viridans*.



**Fig. S4.** Mitotic chromosomes of *Eleocharis*, showing different chromosome numbers and karyotype organisation. A. *Eleocharis ramboana*. Sample 2 with  $2n = 29$ , including one large chromosome. B–E. *E. niederleinii*. B. Sample 14 with  $2n = 35$  and chromosomes of similar size. C. Sample 17 with  $2n = 40$  and two large chromosomes. D. Sample 18 with  $2n = 40$  including two large and one intermediate-sized chromosome. E. Sample 12 with  $2n = 31$  and one large chromosome. F–H. *E. viridans*. F. Sample 26 with  $2n = 40$  and three large chromosomes. G. Sample 20 with  $2n = 20$  and chromosomes of similar size. H. Sample 24 with  $2n = 29$ , including one large chromosome. Arrows indicate the large chromosomes. Scale bar: 10  $\mu\text{m}$ .



**Fig. S5.** *In situ* hybridisation with 35S rDNA probe. A–D. *Eleocharis niederleinii*. A Sample 17 with  $2n = 40$  and four hybridisation signals. B. Sample 16 with  $2n = 40$  and four hybridisation signals. C. Sample 14 with  $2n = 35$  and six hybridisation signals. D. Sample 19 with  $2n = 40$  chromosomes and six hybridisation signals. E, G–I. *E. viridans*. E. *E. viridans*. Sample 20 with  $2n = 20$  and 4 hybridisation signals. G. Sample 25 with  $2n = 31$  and six hybridisation signals. H. Sample 26 with  $2n = 40$  and four hybridisation signals. I. Sample 27 with  $2n = 40$  and six hybridisation signals. F. *E. ramboana*. Sample 2 with  $2n = 29$  and six hybridisation signals. Note that, except where one large chromosome exhibits no terminal FISH signal (arrow in Fig. S4D), all large chromosomes have hybridisation signals. Scale bar: 10  $\mu\text{m}$ .

**Table S1. Synopsis of the morphological features that were used for identification of the samples of *Eleocharis niederleinii*, *E. ramboana* and *E. viridans* studied here**

Information were obtained from Trevisan and Boldrini (2008) and Trevisan and Boldrini (2010)

Morphological feature	<i>E. niederleinii</i>	<i>E. ramboana</i>	<i>E. viridans</i>
<b>Culms</b>			
Base	Thin, non-ligneous	Thin, non-ligneous	Somewhat ligneous, forming a caudex
Width (mm)	0.2–0.8	0.1–0.2	0.3–1.0
<b>Upper sheath</b>			
Colour at the base	Vinaceous	Stramineous	Vinaceous
Length (mm)	1.1–3.5	–	1.5–8.0
Apex shape	Oblique	Slightly oblique	Oblique
Mucron on apex	Absent	Absent or short	Absent or short
<b>Spikelets</b>			
Size (mm)	4.5–8.0 × 1.0–2.0	4.0–5.0 × 1.0–1.8	6.0–13.0 × 2.0–3.0
Shape	Lanceoloid	Ellipsoid or lanceoloid	Ovoid to lanceoloid
Colour of glumes	Vinaceous to dark brown	Translucent white to stramineous	Castaneous to vinaceous with hyaline margin
Floral glumes	Non-imbricated		Imbricated
Flower number	10–18	10–15	30–60
Perianth bristles		0–2, rudimentary	(0)3–5
<b>Achene</b>			
Length (mm)	0.9–1.3	0.8–0.9	1.0–1.5
Width (mm)	0.6–0.7	0.5–0.6	0.6–1.0

**Table S2. Results of fluorescent *in situ* hybridisation (FISH) with 35S rDNA probe in samples of *Eleocharis niederleinii*, *E. ramboana* and *E. viridans***

Numbers in parentheses indicate the larger chromosomes (LC). Ribosomal DNA, 35S rDNA sites number. LC-FISH, LC carrying fluorescent *in situ* hybridisation (FISH) signals

Species sample; 2n; locality	Ribosomal DNA	LC-FISH	Figure number
<i>E. niederleinii</i>			
1; 2n = 20 (0); Alexandrita, MG	4	0	4A
10; 2n = 30 (2); Sapopema, PR	6	2	4D
12; 2n = 31 (1); Tibagi, PR	6	2	4E
14; 2n = 35 (0); Pinhão, PR	6	0	S4C
16; 2n = 40 (0); Alexandrita, MG	4	0	S4B
17; 2n = 40 (2); Biturama, PR	4	2	S4A
19; 2n = 40 (3); Pinhão, PR	6	2	S4D
<i>E. ramboana</i>			
2; 2n = 29 (1); Tibagi, PR	6	0	S4F
9; 2n = 30 (0); Tibagi, PR	6	0	4C
<i>E. viridans</i>			
20; 2n = 20 (0); Viamão, SC	4	0	S4E
24; 2n = 29 (1); Irani, SC	4	1	4B
25; 2n = 31 (2); Caçador, SC	6	2	S4G
26; 2n = 40 (3); Lebon Regis, PR	4	2	S4H
27; 2n = 40 (4); Pinhão, PR	6	3	S4I
30; 2n = 41 (2); Caçador, SC	4	2	4H
31; 2n = 41(4); Balneário Pinhal, RS	6	3	4F
33; 2n = 42 (4); Lapa, PR	6	4	4G

## References

- Nei M, Li WH (1979) Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proceedings of the National Academy of Sciences of the United States of America* **76**, 5269–5273. [doi:10.1073/pnas.76.10.5269](https://doi.org/10.1073/pnas.76.10.5269)
- Trevisan R, Boldrini II (2008) O gênero *Eleocharis* R.Br. (Cyperaceae) no Rio Grande do Sul, Brasil. *Revista Brasileira de Biociências* **6**, 7–67.
- Trevisan R, Boldrini II (2010) Novelties in *Eleocharis* ser. *Tenuissimae* (Cyperaceae), and a key to the species of the series occurring in Brazil. *Systematic Botany* **35**, 504–511. [doi:10.1600/036364410792495836](https://doi.org/10.1600/036364410792495836)