Telomeric sequence detection using FISH

The products obtained using the previously published protocol (Ijdo *et al*, 1991)were too large (>10Kb) and resulted in heavy precipitation on FISH slides. Size of products was reduced to 200-500bp by increasing the primer concentration from 0.1µM to 10µM. Labeling was carried out in the same reaction by substituting 0.1mM of dTTP for fluorescin-12-dUTP or biotin-16-dUTP (Roche).

Eigenmannia sp.1 and sp.2 slides were prepared following Henegariu et al. (2001) and treated with 100µL of RNAse A (100µg/mL in 2xSSC) for 1h at 37ºC, washed in PBS (2x5min) and dehydrated in ethanol. Pepsin treatment (0.005% in 10mM HCl) was carried out for 10min at 37ºC. The slides were then washed (2x5min in PBS) and fixed with 1% formaldehyde (in PBS, 50mM $MgCl_2$) for 15min and washed with PBS (2x5min). Denaturing was performed at 65 ºC for 2min in 70% formamide (in 0.66xSSC), after which the slides were dehydrated in ethanol and air dried.

Slides were covered with 12µL of either a low or high stringency hybridization solution consisting of 1-2µL (50-100ng) labeled probe, 70% (high stringency) or 50% (low stringency) of formamide, 10% dextran sulfate and 2xSSC. The hybridization solution was denatured for 10min at 75ºC. Hybridization took place overnight at 37ºC, and the slides were then washed at 42ºC in 2xSSC containing 50% formamide (2x5min) and 2xSSC. When biotin labeled probes were used, slides were incubated at 37ºC in 100mM Tris-HCl (pH 7.5), 150mM NaCl, 0.5% m/v dry milk for 30min, followed by incubation in the same conditions but with the addition of 10µL Cy3. Slides

were then washed 3 times for 5min in 4xSSC containing 0.005% tween 20 and then dehydrated in ethanol and air-dried. Slides prepared with directly labeled probes were washed 3 times for 5min with 4xSSC containing 0.005% Tween 20, dehydrated and air-dried following overnight hybridization. Slides were counter-stained with DAPI using Vectashield® Mounting Medium with DAPI (Vector Labs., CA, USA) and analyzed with the ImagePro Plus® software. The results are displayed in the figure below (Figure S3.1).

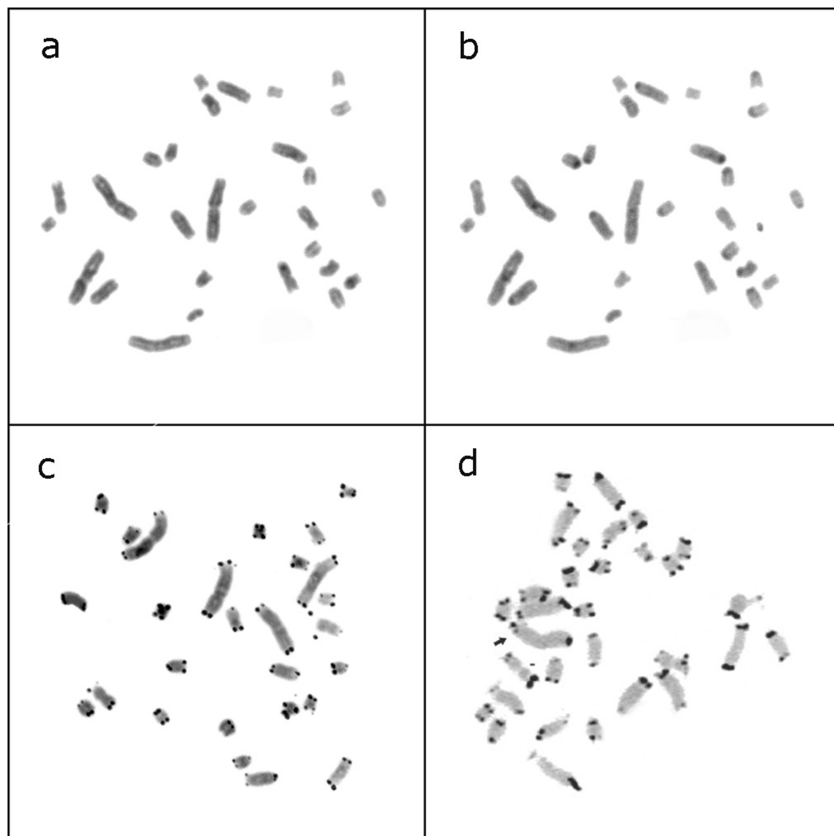

Figure S3.1. Chromomycin-DAPI double staining of Eigenmannia sp.1. using **a)** DAPI and **b)** chromomycin. Results of telomeric sequence detection using FISH on **c)** *Eigenmannia* sp.1 and; **d)** *Eigenmannia* sp.2. The Y chromosome is shown with an arrow in **d)**.

Cited References

Henegariu O, Heerema NA, Lowe Wright L, Bray-Ward P, Ward DC, Vance GH (2001). Improvements in cytogenetic slide preparation: controlled chromosome spreading, chemical aging and gradual denaturing. *Cytometry* **43**(2)**:** 101-109.

Ijdo JW, Wells RA, Baldini A, Reeders ST (1991). Improved telomere detection using a telomere repeat probe (TTAGGG)n generated by PCR. *Nucleic Acids Res* **19**(17)**:** 4780.