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# Identity and origin of the *Campylopus* (Leucobryaceae, Bryopsida) species from Trindade Island (Brazil)

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**Abstract** – Two species of *Campylopus*, *C. fragiliformis* and *C. pilifer*, were previously reported from Trindade Island, a Brazilian island situated in the South Atlantic Ocean around 1100 km off the coast of mainland Brazil. Phylogenetic inferences from sequences of the nuclear ribosomal internal transcribed spacers and three plastid markers are consistent with the recognition of two *Campylopus* species on Trindade. However, the island populations of *C. pilifer* are nested among those of *C. introflexus* and those of *C. fragiliformis* among those of *C. occultus*. A re-investigation of morphological characters confirms that the populations from Trindade Island belong to these species. Based on phylogenetic relationships, both *C. introflexus* and *C. occultus* probably reached Trindade from continental South America. The colonization of Trindade by *C. introflexus* in particular might have been facilitated by the severe human impact on the original vegetation throughout the last five centuries.

***atp*I-*atp*H / *Campylopus introflexus* / *Campylopus occultus* / Island colonization / South Atlantic Ocean**

**Résumé** – Deux espèces de *Campylopus*, *C. fragiliformis* et *C. pilifer*, ont été reportées sur l'île brésilienne de Trindade, dans l'océan Atlantique Sud, à environ 1 100 km des côtes brésiliennes. Les inférences phylogénétiques basées sur des séquences d'ADN des espaceurs transcrits internes (ITS) de l'unité ribosomique et de trois marqueurs chloroplastidiens sont en accord avec la reconnaissance de deux espèces de *Campylopus* sur l'île de Trindade. Néanmoins, les populations insulaires de *C. pilifer* sont imbriquées parmi *C. introflexus* et celles de *C. fragiliformis* parmi *C. occultus*. Le réexamen des caractéristiques morphologiques confirme que les populations de l'île de Trindade appartiennent à ces deux espèces. Sur la base des relations phylogénétiques *C. introflexus* et *C. occultus* ont probablement atteint Trindade à partir de l'Amérique du Sud continentale. L'introduction sur Trindade de *C. introflexus*, en particulier, aurait été favorisée par l'impact humain sévère sur la végétation d'origine durant les cinq derniers siècles.

**Atlantique Sud / *atp*I-*atp*H / *Campylopus introflexus* / *Campylopus occultus* / Colonisation insulaire**

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Trindade is a Brazilian island in the South Atlantic Ocean that arose around three Mya due to volcanic activity (Serafini *et al.*, 2010). As with other Atlantic islands, Trindade had a complex human colonization history from the time of its discovery in 1502 by the Portuguese sailor João da Nova. After having been used by pirates and slave traders, colonization attempts by the British and Azoreans in the 18th century impacted the island the most, especially because of the introduction of goats and other animals that destroyed the original vegetation almost completely (Silva & Alves, 2011; Faria *et al.*, 2012).

The extant bryophyte flora of Trindade comprises 32 species, 20 liverworts, 11 mosses and one hornwort (Faria *et al.*, 2012). Biogeographic affinities are strongest with continental Brazil, more specifically with the fragments of Atlantic Rainforest of the Southeast Region of Brazil, with which Trindade shares 96% of its bryoflora (Faria *et al.*, 2012). However, the strong historical human impact and the possible function of Trindade as a bridge between the Old and New World, might have led to more diverse origins of the actual bryophyte populations found on the island. Although the bryoflora of Trindade is already quite well-known, the identity of species of large and taxonomically complex genera such as *Campylopus* Brid. (*cf.* Frahm, 1980, 1987, 1988, 1990, 1991; Stech, 2004; Stech & Dohrmann, 2004; Stech & Wagner, 2005; Stech *et al.*, 2010) may need further study. Faria *et al.* (2012) included two taxa of *Campylopus* in their inventory of Trindade Island, namely *C. fragilis* subsp. *fragiliformis* (J.-P. Frahm) J.-P. Frahm and *C. pilifer* Brid. The former is endemic to the Atlantic Rainforest in Brazil and was originally considered a separate species, *C. fragiliformis* J.-P. Frahm, a hypothesis consistent with inferences from molecular data (Stech *et al.*, 2010). *Campylopus pilifer* is a mainly tropical taxon found in tropical America, Africa, parts of the Middle East and Asia, extending to Europe and North America, with the New World and Old World populations composing two distinct molecular lineages (Stech & Dohrmann, 2004).

The *Campylopus* populations on Trindade Island exhibit peculiar morphologies when compared to their continental counterparts. Island plants of *C. pilifer* are strikingly smaller than those found on the continent and exhibit shorter dorsal lamellae on the costa. The specimens of *C. fragiliformis* were found bearing comal tufts, a trait not known from this species (*cf.* Frahm, 1979).

Recent molecular phylogenetic studies have helped to clarify systematic and biogeographic relationships of island populations in the genus *Campylopus* (Stech *et al.*, 2007, 2010) and bryophytes in general (Sim-Sim *et al.*, 2005; Stech *et al.*, 2008; Laenen *et al.*, 2011; Patiño *et al.*, 2013a, b; Draper *et al.*, 2015). However, no molecular study is available yet for South Atlantic islands such as Trindade.

Here we seek to confirm the identity of the *Campylopus* taxa occurring on Trindade and infer the geographic origins of the island populations, based on analyses of nuclear (ITS) and chloroplast (*atp*B-*rbc*L, *atp*I-*atp*H and *trn*L-*trn*F) markers and morphology.

## MATERIALS AND METHODS

### Sampling

A molecular dataset comprising sequences of the *atp*B-*rbc*L spacer, *trn*L-*trn*F region and ITS for 58 specimens of *Campylopus* and two specimens of *Pilopogon* Brid., used as outgroup representatives, was compiled from Stech & Dohrmann (2004) and Stech *et al*. (2010). Fourteen new specimens were sequenced for the present study, for which voucher information and GenBank accession numbers are given (Table 1). In addition, multiple plastid markers (*atp*I-*atp*H, *psb*A-*trn*H, *rps*7-*rps*12, *trn*S-*rps*4, *rps*4-*trn*T) were tested for efficiency of amplification and sequencing, as well as sequence variability. Based on those criteria, *atp*I-*atp*H spacer was selected and newly sequenced for the present study. GenBank accession numbers for the *atp*I-*atp*H sequences of the specimens not listed in Table 1 are KU163078-KU163123. For voucher information of these specimens see Stech & Dohrmann (2004) and Stech *et al*. (2010).

## DNA extraction, amplification and sequencing

Genomic DNA was extracted using the NucleoSpin® Plant II Kit (Macherey-Nagel, Düren, Germany). PCR amplification protocols followed Gama *et al*. (2015) for nuclear and plastid markers.

New primers were designed for the newly investigated *atp*I-*atp*H spacer based on *Campylopus introflexus* sequences: *atp*I_F 5'-ACAGACGCAGCAGAAA TTAAGG-3' and *atp*H_R 5'-AGGGGAATCGTTAGAAGGTCAT-3', using the plugin – Primer3 (Koressar & Remm, 2007) implemented in Geneious R8 (Kearse *et al*., 2012). Sequencing was performed at Macrogen Inc.

## Phylogenetic reconstruction

Sequences were aligned using MAFFT (Katoh *et al*., 2002) embedded in Geneious R8 and manually adjusted and the loci concatenated. Gaps were treated as informative by simple indel coding (SIC) (Simmons & Ochoterena, 2000) using SeqState (Müller, 2004). All alignment positions and gaps were included in the analyses. Phylogenetic analyses were performed under maximum likelihood (ML) and Bayesian inference (BI). The GTR + Γ model, selected under the Akaike information criterion in jModelTest2 (Guindon & Gascuel, 2003; Darriba *et al*., 2012), was applied in the model-based approaches. Separate analyses with each of the loci were performed to check for incongruences. Maximum likelihood trees were calculated using RaxML v8.0.26 (Stamatakis, 2014) through the raxmlGUI v.1.3.1 (Silvestro & Michalak, 2012). Bootstrap support (BS) values were obtained with a thorough bootstrap algorithm and 10,000 pseudoreplicates. Bayesian analyses were run using MrBayes v3.2.5 (Ronquist *et al*., 2012) on the CIPRES Science Gateway v3.1 (Miller *et al*., 2010). Bayesian posterior probabilities (PP) were estimated by the Markov Chain Monte Carlo (MCMC) method. Default prior probabilities for all model parameters were used as implemented in the software. Four runs with four chains each (three heated and one cold) were run with $30 \times 10^6$ generations, chains sampled every 1,000th generation and the respective trees written to a tree file. A threshold of $< 0.01$ for the standard deviation of split frequencies was used to assess convergence of runs. Fifty percent majority rule consensus trees and posterior probabilities of clades were calculated combining the four runs using the trees sampled after the convergence of the chains and the "burnin" (25% of the trees) discarded.

Table 1. Voucher information and GenBank accession numbers for the newly generated sequences of *Campylopus introflexus* (Hedw.) Brid. and *C. occultus* Mitt.

| *Specimen* | *Original identification* | *Voucher* | *Herbarium* | *Country – Locality* | *GenBank acc. no.* | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | *atp*B-*rbc*L | *atp*I-*atp*H | ITS | *trn*L-*trn*F |
| *C. introflexus* 01 | – | 2 Oct. 2003, *Frahm s.n.* | BONN, L | France – Dept. Finistère | – | KU163121 | KU163124 | – |
| *C. introflexus* 02 | *C. incrassatus* Müll. Hal. | *Streimann 49976* | BONN, L | Australia – NSW | – | KU163083 | KU163125 | KU212887 |
| *C. introflexus* 03 | – | *Bordin 129* | HUCS | Brazil – Rio Grande do Sul | KU163137 | KU163078 | KT985061 | KU212888 |
| *C. introflexus* 04 | *C. pilifer* Brid. | *Faria 227* | UB | Brazil – Trindade Island | – | KU163117 | KU163126 | – |
| *C. introflexus* 05 | *C. pilifer* | *Faria 77* | UB | Brazil – Trindade Island | – | KU163113 | KU163127 | KU212889 |
| *C. introflexus* 06 | *C. pilifer* | *Faria 66* | UB | Brazil – Trindade Island | – | KU163116 | KU163128 | – |
| *C. introflexus* 07 | *C. pilifer* | *Faria 102* | UB | Brazil – Trindade Island | – | KU163112 | KU163129 | – |
| *C. introflexus* 08 | *C. pilifer* | *Faria 848* | UB | Brazil – Trindade Island | – | KU163114 | KU163130 | – |
| *C. introflexus* 09 | *C. pilifer* | *Faria 76* | UB | Brazil – Trindade Island | – | KU163115 | KU163131 | – |
| *C. introflexus* 10 | *C. pilifer* | *Faria106* | UB | Brazil – Trindade Island | – | – | KU163132 | KU212890 |
| *C. occultus* 01 | *C. flexuosus* (Hedw.) Brid. | *Müller R284* | BONN, L | France – Réunion Island | – | KU163120 | KU163133 | – |
| *C. occultus* 02 | *C. fragilis* J.-P. Frahm | *Faria 610* | UB | Brazil – Trindade Island | – | – | KU163134 | KU212891 |
| *C. occultus* 03 | – | *Yano & Peralta 32359* | SP | Brazil – São Paulo | – | KU163094 | KU163135 | KU212892 |
| *C. occultus* 04 | – | *Yano & Peralta 31880* | SP | Brazil – São Paulo | – | KU163099 | KU163136 | KU212893 |

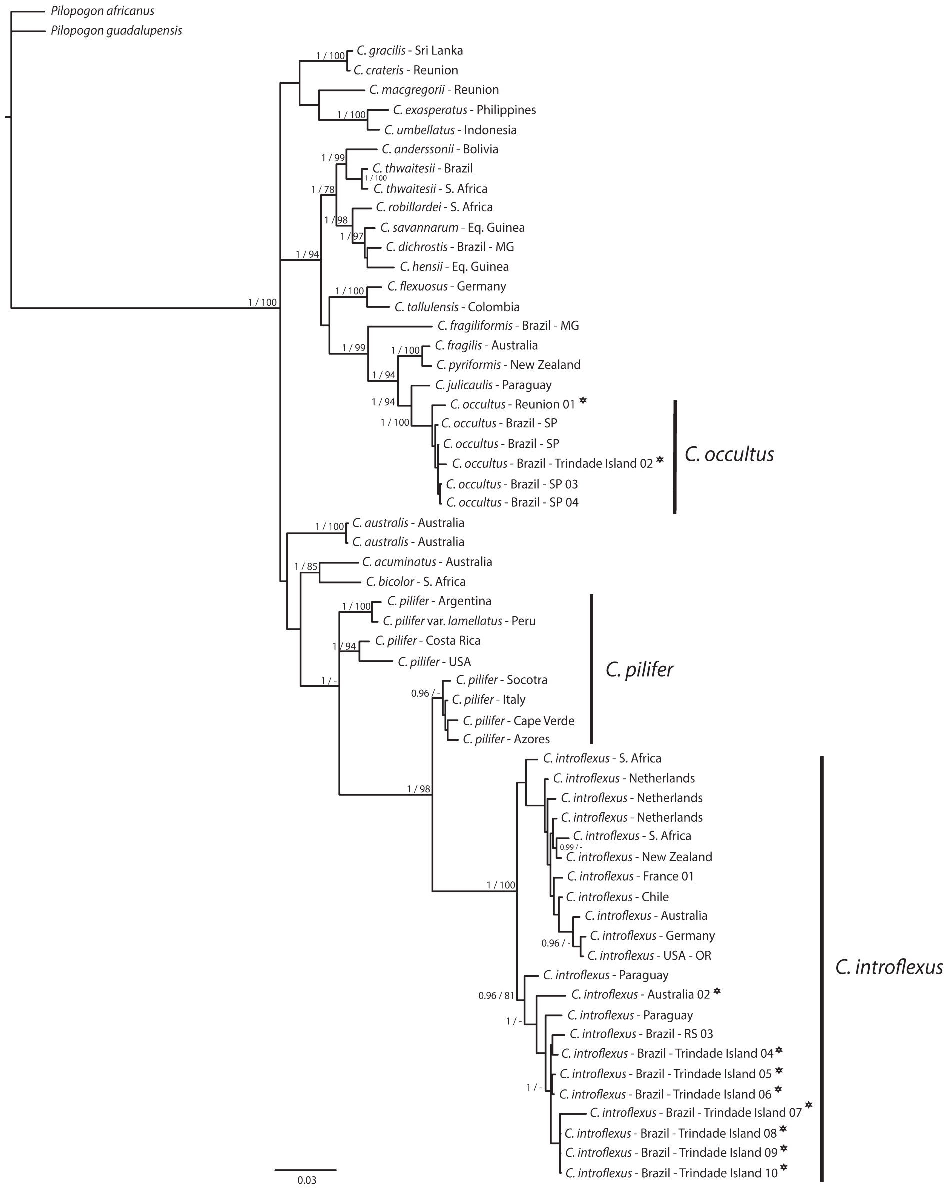

Fig 1. Phylogram obtained from Bayesian analysis of a combined dataset of nrITS, plastid regions (*atp*B-*rbc*L, *atp*I-*atp*H, *trn*L-*trn*F) and indels coded by simple indel coding. Numbers at the branches are Bayesian posterior probabilities ≥ 0.95 over bootstrap support values ≥ 75% from maximum likelihood analysis of the same dataset. The symbol "☆" denotes specimens that were originally identified as belonging to a different species (for original identifications see Table 1).

### Morphological analysis

In order to confirm the identity of the specimens from Trindade Island, the morphological study was based on specimens that were included in the molecular analyses and additional specimens from the following herbaria: BM, HUCS, L, MO, PC, S, SP, U, and UB. Specimens were rehydrated and mounted on a slide with Hydromatrix solution (Micro Tech Lab, Graz, Austria) for microscopic observations. Slide preparation was carried out using a stereoscope and detailed observations of plant material were done using a light microscope. Diagnostic traits (i.e.: habit, plant size, leaf size and shape, lamina cell shape, length and width of costa and costal anatomy in cross-section) used to characterize morphological taxa were defined based on literature (Frahm, 1991; Frahm & Stech, 2006).

## RESULTS

The combined nuclear and plastid alignment including indels comprised a total of 4111 characters (ITS1 1-1076, 5.8S rRNA gene 1077-1251, ITS2 1252-1817, *atp*B-*rbc*L 1818-2472, *atp*I-*atp*H 2473-2871, *trn*L-*trn*F 2872-3391, and indels 3392-4111). No incongruence with respect to well-supported clades (≥ 0.95 for BI and ≥ 75% for ML) was found in separate analyses of the different loci. The concatenated dataset was used for phylogenetic analysis under Bayesian Inference (BI) and Maximum Likelihood (ML). The Bayesian consensus tree and the optimal maximum likelihood tree (not shown) resolved the same major clades. Within *Campylopus* three main clades of ambiguous relationships were resolved. The first clade includes five species from South East Asia and the Indian Ocean and lacks support from BI and ML analyses. The second clade includes many (sub)tropical and southern temperate species and is well supported. *Campylopus fragiliformis* belongs to this clade but the two specimens are resolved in two clades: the specimen from Trindade Island is part of the *C. occultus* clade (PP 1, BS 100%), whereas the specimen from mainland Brazil is resolved as sister to the clade comprising *C. fragilis*, *C. pyriformis* (Schultz) Brid., *C. julicaulis* Broth. and *C. occultus* (PP 1, BS 99%). Within the third clade, *C. pilifer* is resolved as paraphyletic with two clades of New World populations, and one clade comprising all Old World specimens. All specimens of *C. pilifer* from Trindade island are nested among those of *C. introflexus*, with which they compose a maximally supported clade (PP1, BS 100%). This *C. introflexus* clade comprises two recognizable lineages, one of which with strong support (PP 0.96, BS 81%) which includes the Trindade samples. Sequences of the island specimens within the *C. introflexus* clade are almost identical to those of continental Brazil.

## DISCUSSION

Phylogenetic inferences from DNA sequences are consistent with the recognition of two species of *Campylopus* on Trindade Island. However, in both cases the original identification of the sequenced specimens did not fit with their phylogenetic position. In contrast to what was previously known (Faria *et al*., 2012), *C. pilifer* on Trindade belongs to *C. introflexus*, and *C. fragiliformis* to *C. occultus*.

*Campylopus pilifer* typically has dorsal costal lamellae that are three up to seven cells high and leaves ending in a straight hyaline hairpoint. By contrast, the costal lamellae of *C. introflexus* are not higher than two cells on the highest point and the hyaline hairpoint, when present, is strongly recurved (ca. 90°). In *C. pilifer* var. *brevirameu*s, the costal lamellae resemble those of *C. introflexus*, with 2-3 cells on the highest point, however, the hairpoints are always straight, and based on DNA data such plants are clearly resolved as *C. pilifer* (Frahm & Stech, 2006). A careful morphological investigation showed that the specimens from Trindade molecularly identified as *C. introflexus* display the characteristics of *C. introflexus*: costal lamellae with no more than two cells and reflexed hairpoints. However, some plants within a tuft lacked the hyaline hairpoint, which could be due to microclimatic factors such as different degrees of light exposure. When compared to *C. pilifer*, the Trindade specimens are strikingly smaller and have a dull green color. They could thus have been, superficially, mistaken for young specimens of *C. pilifer*.

The current natural distribution of *C. introflexus* ranges from Antarctica (beyond 60$^{o}$ South) to subtropical South America, southern Africa, several Atlantic islands northwards to St. Helena, Australia and New Zealand (Stech & Dohrmann, 2004; Klinck, 2010). Although the distribution map of Klinck (2010) includes southern mainland Brazil as part of the distribution of *C. introflexus*, the species was not listed in the latest checklist of the bryophytes of Brazil (Costa *et al.*, 2011). The present data, however, confirm that *C. introflexus* occurs both in mainland Brazil and Trindade Island. Both areas are well within the natural distribution of *C. pilifer* (cf. Stech & Dohrmann, 2004) but at its northern limit, which probably contributed to the misconception of *C. introflexus* in Brazil, and Trindade in particular.

The *C. introflexus* specimens found on Trindade Island form a well-supported clade together with a specimen from southern continental Brazil, with which they share almost identical DNA sequences (less than 0.5% difference). This suggests that the populations from the island could have originated from a South American continental ancestor. While southern continental Brazil shows a climate suitable for *C. introflexus*, with warm-temperate, fully humid climate and mild summers (type "Cf" in the Köppen-Geiger climate classification; Kottek *et al.*, 2006), Trindade has a more arid climate. However, the species possibly benefited from the human impact on Trindade, since *C. introflexus* is adapted to both natural and man-made disturbed habitats (Hassel & Söderström, 2005; Hasse, 2007; Sparrius & Kooijman, 2011; Carter, 2014). The ecological conditions together with the restricted morphological and molecular variation within *C. introflexus* on the island suggest that the species may have colonized Trindade in a single, recent event. However, further data, including a dated phylogeny, is needed in order to test this hypothesis.

Specimens molecularly identified as *C. occultus* are morphologically identified by a combination of characters, including hyaline and not inflated alar cells, quadrate to oblate basal marginal laminal cells, incrassate upper laminal cells, and a costa occupying ⅓ of the leaf width, showing a ridged back in transverse section, with ventral hyalocysts and dorsal stereids. By contrast, *C. fragiliformis* has inflated hyaline alar cells and basal hyaline basal cells extending up to midleaf, rectangular laminal cells above the alar cells, a costa occupying ½ to ⅔ of the leaf width, and with a smooth dorsal surface in cross-section. As well, *C. occultus* is often found with comal tufts, which is lacking in *C. fragiliformis*.

*Campylopus occultus* was previously confined to SE Brazil, Uruguay, Paraguay and northern Argentina. Its distribution is hereby shown to extend eastwards to Trindade Island and Réunion in the Indian Ocean. Frahm & Stech (2006) and

Stech *et al*. (2010) show a similar disjunct distribution including Réunion and the New World in the New World *C. pilifer* clade. Whether this pattern is caused by long distance dispersal from the Neotropics to Réunion or by human introduction in recent history (last 500 years) needs to be further investigated. Similarly, the exact geographic origin of the population of *C. occultus* found on Trindade Island needs to be inferred from more extensive sampling across its continental South American distribution.

The present study emphasizes the need of a comprehensive morpho-molecular revision in order to resolve taxonomic problems and incongruences in species delimitations, and refine species identification in the speciose and taxonomically complex genus *Campylopus*. *Campylopus introflexus* and *C. pilifer* show how the reassessment of intraspecific morphological and molecular variation of species can clarify natural distributions and indicate where further investigation is needed.

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## REFERENCES

CARTER B.E., 2014 — Ecology and distribution of the introduced moss *Campylopus introflexus* (Dicranaceae) in western North America. *Madroño* 61: 82-86.

COSTA D.P., PÔRTO K.C., LUIZI-PONZO A.P., ILKIU-BORGES A.L., BASTOS C.J.P., CÂMARA P.E.A.S., PERALTA D.F., BÔAS-BASTOS S.B.V., IMBASSAHY C.A.A., HENRIQUES D.K., GOMES H.C.S., ROCHA L.M., SANTOS N.D., SIVIERO T.S., VAZ-IMBASSAHY T.F. & CHURCHILL S.P., 2011 — Synopsis of the Brazilian moss flora: checklist, distribution and conservation. *Nova Hedwigia* 93: 277-334.

DARRIBA D., TABOADA G.L., DOALLO R. & POSADA D., 2012 — jModelTest 2: more models, new heuristics and parallel computing. *Nature methods* 9: 772.

DRAPER I., HEDENÄS L., STECH M., PATIÑO J., WERNER O., GONZÁLEZ-MANCEBO J.M., SIM-SIM M., LOPES T. & ROS R.M., 2015 — How many species of *Isothecium* (Lembophyllaceae, Bryophyta) are there in Macaronesia? A survey using integrative taxonomy. *Botanical journal of the Linnean society* 177: 418-438.

FARIA A.L.A, CARVALHO-SILVA M., COSTA D.P. & CÂMARA P.E.A.S., 2012 — The bryophytes of Trindade Island, South Atlantic, Brazil. *Acta botanica Brasilica* 26: 783-793.

FRAHM J.-P., 1979 — Die Campylopus-Arten Brasiliens. *Revue bryologique et lichénologique* 45: 127-178.

FRAHM J.-P., 1980 — Synopsis of the genus *Campylopus* in North America north of Mexico. *The bryologist* 83: 570-588.

FRAHM J.-P., 1984 — Phytogeography of European *Campylopus* species. *Proceedings of the III. Meeting of Bryologists from Central and East Europe*, Prague, pp. 191-212.

FRAHM J.-P., 1987 — A revised list of the *Campylopus* species of the world. *Bryologische Beiträge* 7: 1-117.

FRAHM J.-P., 1988 — The subantarctic and southern hemispheric species of *Campylopus* (Dicranaceae), with contributions to the origin and speciation of the genus. *Journal of the Hattori botanical laboratory* 64: 367-388.

FRAHM J.-P., 1990 — *Campylopus*, a modern and successful genus!? *Tropical bryology* 2: 91-101.

FRAHM J.-P., 1991 — Campylopodioideae, Paraleucobryoideae. *Flora neotropica monographs* 54: 1-238.

FRAHM J.-P. & STECH M., 2006 — The taxonomic status of intermediate forms of *Campylopus introflexus* (Hedw.) Brid. and *C. pilifer* Brid. (Dicranaceae, Bryopsida) newly discovered in Europe. *Cryptogamie, Bryologie* 27: 213-223.

GAMA R., STECH M., SCHÄFER-VERWIMP A. & PERALTA D.F., 2015 — A morpho-molecular revision of *Leucoloma* (Dicranaceae, Bryophyta) in Brazil. *Nova Hedwigia* 100: 319-332.

GUINDON S. & GASCUEL O., 2003 — A simple, fast and accurate method to estimate large phylogenies by maximum-likelihood. *Systematic biology* 52: 696-704.

HASSE T., 2007 — *Campylopus introflexus* invasion in a dune grassland: Succession, disturbance and relevance of existing plant invader concepts. *Herzogia* 20: 305-315.

HASSEL K. & SÖDERSTRÖM L., 2005 — The expansion of the neophytes *Orthodontium lineare* and *Campylopus introflexus* in Britain and continental Europe. *Journal of the Hattori botanical laboratory* 97: 183-193.

KATOH K., MISAWA K., KUMA K. & MIYATA T., 2002 — MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic acids research* 30: 3059-3066.

KEARSE M., MOIR R., WILSON A., STONES-HAVAS S., CHEUNG M., STURROCK S., BUXTON S., COOPER A., MARKOWITZ S., DURAN C., THIERER T., ASHTON B., MENTJIES P. & DRUMMOND A., 2012 — Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28: 1647-1649.

KLINCK J., 2010 — *NOBANIS – Invasive Alien Species Fact Sheet – Campylopus introflexus*. Online Database of the European Network on Invasive Alien Species, www.nobanis.org. Date of access 1/09/2015.

KORESSAAR T. & REMM M., 2007 — Enhancements and modifications of primer design program Primer3. *Bioinformatics* 23: 1289-1291.

KOTTEK M., GRIESER J., BECK C., RUDOLF B. & RUBEL F., 2006 — World Map of the Köppen-Geiger climate classification updated. *Meteorologische Zeitschrift* 15: 259-263.

LAENEN B., DÉSAMORÉ A., DEVOS N., SHAW A.J., GONZÁLEZ-MANCEBO J.M., CARINE M.A. & VANDERPOORTEN A., 2011 — Macaronesia: a source of hidden genetic diversity for post-glacial recolonization of western Europe in the leafy liverwort *Radula lindenbergiana*. *Journal of biogeography* 38: 631-639.

MILLER M.A., PFEIFFER W. & SCHWARTZ T., 2010 — Creating the CIPRES Science Gateway for inference of large phylogenetic trees. *Proceedings of the Gateway Computing Environments Workshop (GCE)*, New Orleans, LA, pp. 1-8.

MÜLLER K., 2004 — SeqState - primer design and sequence statistics for phylogenetic DNA data sets. *Applied bioinformatics* 4: 65-69.

PATIÑO J., BISANG I., HEDENÄS L., DIRKSE G., BJARNASON Á.H., AH-PENG C. & VANDERPOORTEN A., 2013a — Baker's law and the island syndromes in bryophytes. *Journal of ecology* 101: 1245-1255.

PATIÑO J., CARINE M., FERNÁNDEZ-PALACIOS J.M., OTTO R., SCHAEFER H. & VANDERPOORTEN A., 2013b — The anagenetic world of spore-producing land plants. *New phytologist* 201: 305-311.

RONQUIST F., TESLENKO M., MARK P.V.D., AYRES D.L., DARLING A., HÖHNA S., LARGET B., LIU L., SUCHARD, M.A. & HUELSENBECK J.P., 2012 — MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic biology* 61(3): 539-542.

SERAFINI T.Z., FRANÇA G.B. & ANDRIGUETTO-FILHO J.M., 2010 — Ilhas oceânicas brasileiras: biodiversidade conhecida e sua relação com o histórico de uso e ocupação humana. *Journal of integrated coastal zone management* 10: 281-301.

SILVA N.G. & ALVES R.J.V., 2011 — The eradication of feral goats and its impact on plant biodiversity – a milestone in the history of Trindade Island, Brazil. *Rodriguésia* 62: 717-719.

SILVESTRO D. & MICHALAK I., 2012 — raxmlGUI: a graphical front-end for RaxML. *Organisms diversity and evolution* 12: 335-337.

SIM-SIM M., ESQUÍVEL M.G., FONTINHA S. & STECH M., 2005 — The genus *Plagiochila* (Plagiochilaceae, Hepaticophytina) in Madeira Island: Molecular relationships, ecology, and biogeographic affinities. *Nova Hedwigia* 81: 449-462.

SIMMONS M.P. & OCHOTERENA H., 2000 — Gaps as characters in sequence-based phylogenetic analyses. *Systematic biology* 49: 369-381.

SPARRIUS L. & KOOIJMAN A.M., 2011 — Invasiveness of *Campylopus introflexus* in drift sands depends on nitrogen deposition and soil organic matter. *Applied vegetation science* 14: 221-229.

STAMATAKIS A., 2014 — RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30: 1312-1313.

STECH M., 2004 — Supraspecific circumscription and classification of *Campylopus* (Dicranaceae, Bryopsida) based on inferences from sequence data. *Systematic botany* 29: 817-824.

STECH M. & DOHRMANN J., 2004 — Molecular relationships and biogeography of two Gondwanan *Campylopus* species, *C. pilifer* and *C. introflexus* (Dicranaceae). In: GOFFINET B., HOLLOWELL V. & MAGILL R. (eds.) Molecular systematics of bryophytes. *Monographs in systematic botany from the Missouri Botanical Garden* 98: 415-431.

STECH M. & WAGNER D., 2005 — Molecular relationships, biogeography, and evolution of Gondwanan *Campylopus* species (Dicranaceae, Bryopsida). *Taxon* 54: 377-382.

STECH M., SIM-SIM M. & FRAHM J.-P., 2007 — *Campylopus* (Leucobryaceae, Bryopsida) on Madeira Island - Molecular relationships and biogeographic affinities. *Nova Hedwigia* 131: 91-100.

STECH M., SIM-SIM M. & KRUIJER J.D., 2010 — *Campylopus* Brid. (Leucobryaceae) in Macaronesia revisited. *Tropical bryology* 31: 154-163.

STECH M., SIM-SIM M., ESQUÍVEL M.G., FONTINHA S., TANGNEY R., LOBO C., GABRIEL R. & QUANDT D. 2008 — Explaining the “anomalous” distribution of *Echinodium* Jur. (Bryopsida): independent evolution in Macaronesia and Australasia. *Organisms diversity and evolution* 8: 282-292.