Supplementary information

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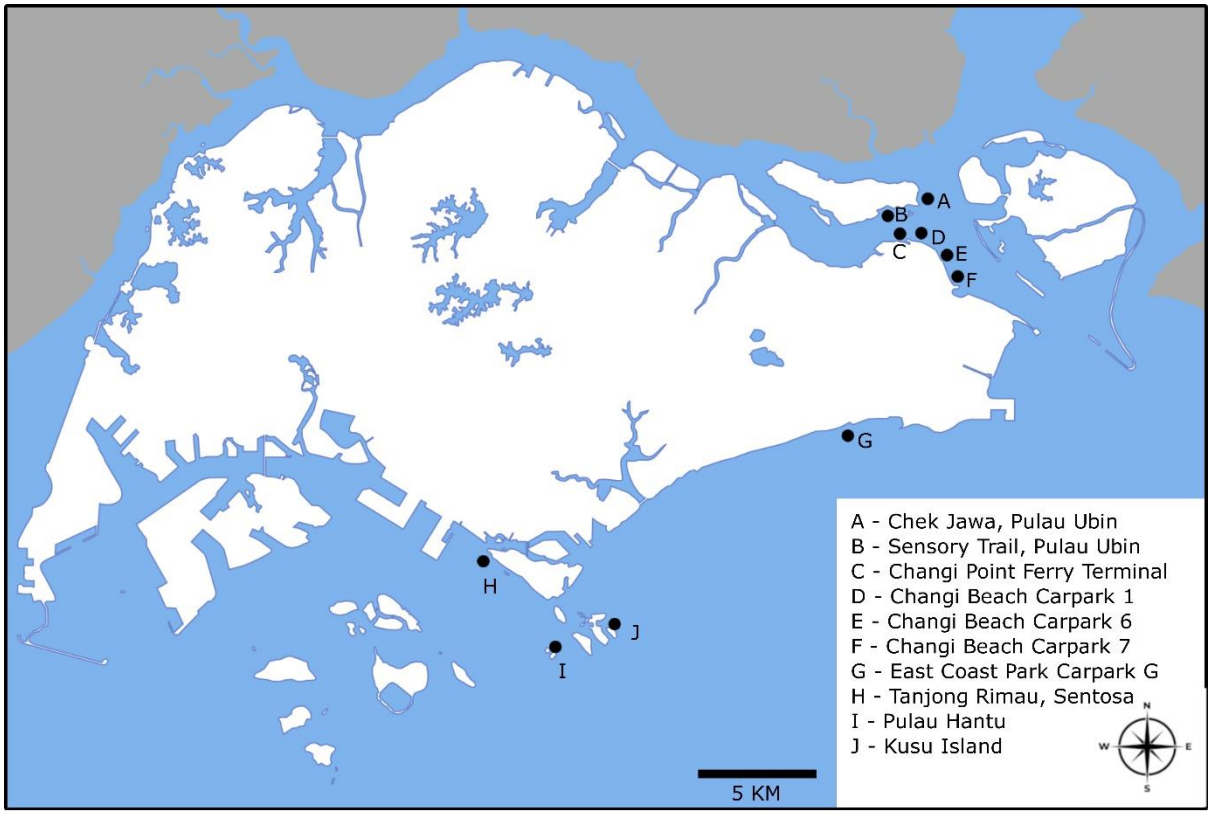

Figure S1: *Gayralia* collection map of Singapore.

Table S1: *Gayralia* GenBank sequences and herbarium vouchers.

Source	Specimen/species	ITS	*tuf*A	Voucher
This study	*Gayralia brasiliensis* (NYF220806)	**OR652592**	**OR597303**	**NYF220806**
	Gayralia brasiliensis (NYF221208)	**OR652593**	-	**NYF221208**
	Gayralia brasiliensis (NYF221213)	**OR652594**	-	**NYF221213**
	Gayralia brasiliensis (NYF230503)	**OR652595**	-	**NYF230503**
Bloomster et al. 2002	*Monostroma grevillei*	**AF499456**	-	
Bast et al. 2015a	*Monostroma grevillei*	**GU062560**	-	
Kawashima et al. 2014	*Gayralia kuroshiensis*	**AB933331**	-	
Bast et al. 2015b	*Gayralia kuroshiensis*	**GU062561**	-	
Bast et al. 2009	*Monostroma latissimum*	**EU664979**	-	
Cui et al. 2022	*Gayralia brasiliensis*	**OP151102**	-	
	Gayralia brasiliensis	**OP151103**	-	
	Gayralia brasiliensis	**OP151104**	-	
	Monostroma sp.	**OP151107**	-	
	Monostroma sp.	**OP151108**	-	
	Monostroma nitidum	**OP151124**	-	
Pellizzari et al. 2013	*Gayralia oxyperma*	**KC143759**	-	
	Gayralia brasiliensis	**KC143761**	-	
	Gayralia brasiliensis (Holotype)	**KC143762**	-	
	Gayralia brasiliensis	**KC143763**	-	
	Gayralia brasiliensis	**KC143764**	-	
	Gayralia brasiliensis	**KC143765**	-	
	Gayralia brasiliensis	**KC143766**	-	
	Gayralia brasiliensis	**KC143767**	-	
	Gayralia brasiliensis	**KC143768**	-	
	Gayralia brasiliensis	**KC143769**	-	
	Gayralia brasiliensis	**KC143770**	-	
Silva et al. 2022	*Gayralia brasiliensis*	**ON312901**	-	
	Gayralia brasiliensis	**ON312902**	-	
	Gayralia brasiliensis	**ON312903**	-	
	Gayralia brasiliensis	**ON312904**	-	
	Gayralia brasiliensis	**ON312905**	-	
	Gayralia brasiliensis	**ON312906**	-	
	Gayralia brasiliensis	**ON312907**	-	
	Gayralia brasiliensis	**ON312908**	-	
	Gayralia brasiliensis	**ON312909**	-	
	Gayralia brasiliensis	**ON312910**	-	
	Gayralia brasiliensis	**ON312911**	-	
	Gayralia brasiliensis	**ON312912**	-	
	Gayralia brasiliensis	**ON312913**	-	
	Gayralia brasiliensis	**ON312914**	-	
Saunders & Kucera 2010	*Monostroma grevillei*	-	**HQ610252**	
	Gayralia oxysperma	-	**HQ610259**	
Weinberger et al. 2019	*Kornmannia leptoderma*	-	**MG944400**	
	Kornmannia leptoderma	-	**MF441479**	
Steinhagen et al. 2018	*Monostroma grevillei*	-	**MH475469**	
Carlile et al. 2011	*Gayralia* sp.	-	**JF680967**	

Source	Specimen/species	ITS	*tuf*A	Voucher
Unpublished	*Gayralia brasiliensis*	**ON400499**	-	
	Gayralia brasiliensis	**KC661349**	-	
	Gayralia brasiliensis	**JF918550**	-	
	Monostroma angicava	**MG572118**	-	
	Protomonostroma sp.	**MG572119**	-	
	Monostroma nitidum	**AY026917**	-	
	Gayralia oxysperma	**AY016306**	-	
	Monostroma nitidum	**AF415170**	-	
	Monostroma articum	**AF415171**	-	
	Monostroma angicava	**AF415173**	-	
	Monostroma angicava	-	**MG646366**	
	Kornmannia leptoderma	-	**MW242793**	
	Gayralia brasiliensis	-	**MW242795**	
	Gayralia brasiliensis	-	**NC_072923**	
	Monostroma nitidum	-	**NC_072924**	

Table S2: Primers and conditions used to amplify *tuf*A and ITS sequences.

Gene	Primers	Sequences (5' to 3')	References
*tuf*A	tufGF4 (forward)	GGN GCN GCN CAA ATG GAY GG	Saunders & Kucera 2010; Kang et al. 2019
	tufAR (reverse)	CCT TCN CGA ATM GCR AAW CGC	Saunders & Kucera 2010; Kang et al. 2019
ITS	18S150F (forward)	TCT TTG AAA CCG TAT CGT GA	Blomster et al. 1998; Kang et al. 2019
	ENT26SA (reverse)	GCT TAT TGA TAT GCT TAA GTT CAG CGG GT	Blomster et al., 1998; Kang et al., 2019

Gene	PCR parameters (35 cycles)
*tuf*A	Initial denaturation: 94°C for 5 min Denaturation: 94°C for 1 min Annealing: 50°C for 1 min Elongation: 72°C for 2 min Final elongation: 72°C for 7 min
ITS	Initial denaturation: 94°C for 5 min Denaturation: 94°C for 1 min Annealing: 55°C for 1 min Elongation: 72°C for 2 min Final elongation: 72°C for 7 min

Detailed molecular methods

Samples were digested in 20 µl of proteinase K and 900 µl of cetyltrimethylammonium bromide (CTAB) (Doyle and Doyle 1987). 25:24:1 phenol-chloroform-isoamyl alcohol was utilised to extract DNA from digested tissues (Doyle and Doyle 1987). Published primers (Table S2) were used to amplify the genes *tuf*A and ITS. GoTaq DNA polymerase (Promega) was used for polymerase chain reaction (PCR). 1% agarose gel electrophoresis was conducted to determine the success of PCR amplification. Afterwards, PCR products were purified utilising Beckman Coulter AMPure XP beads in conjunction with a magnetic plate. Cycle sequencing was performed using BigDye Terminator 5X Sequencing Buffer v3.1, BigDye Terminator v3.1 (Applied Biosystems, Waltham). For DNA precipitation, CleanSEQ Dye Terminator Removal Kit (Beckman Coulter, Brea) was used according to the manufacturer's instructions. ABI 3130XL DNA Analyzer (ThermoFisher Scientific) was used for Sanger sequencing.

To assemble and check the sequencing data, Geneious Prime v2022.0.1 (https://www.geneious.com/prime/) was used. The assembled sequences were identified preliminarily based on the Basic Local Alignment Search Tool (BLAST) against GenBank sequences at the National Centre for Biotechnology Information (NCBI) (Clark et al. 2016, Sayers et al. 2019). Previously published sequences (Table S2) and the newly generated sequences were assembled and aligned in Mesquite v3.70 (Maddison and Maddison 2021) and MAFFT v7.49 (Katoh and Standley 2013) under default parameters. *Protomonostroma* sp. was used as an outgroup in the ITS tree as this genus has been demonstrated to be a closely-related outgroup of *Gayralia* and *Monostroma* (Pellizzari et al. 2013). However, *Monostroma angicava* was used as an outgroup in the *tuf*A tree as the interspecific distance between *Protomonostroma* specimens in GenBank and our specimens were much larger than other distantly related genera like *Acrosiphonia*.

Maximum likelihood (ML) and Bayesian inference (BI) were used for phylogenetic analysis. For ML, RAxML v8.2.4 (Stamatakis 2014) was run under the GTRGAMMA model with 50 random starting trees. One thousand bootstrap pseudoreplicates were used to test clade stability. For BI, jModelTest v2.1.10 (Darriba et al. 2012; Guindon and Gascuel 2003) was used to determine the optimal nucleotide substitution model. GTR+G and GTR+I+G were the best substitution models for *tuf*A and ITS respectively. BI was conducted using MrBayes v3.2.7 (Huelsenbeck et al. 2001; Ronquist et al 2003). Two runs of four Markov chains over 10 million generations were performed with one tree logged after every 100 generations. The initial 10001 trees were discarded as burn-in following trace inspection with Tracer v1.7.2 (Rambaut et al. 2018) and the remaining trees summarised based on majority-rule consensus.

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