Phylogenomics of peacock spiders and their kin (Salticidae: *Maratus*), with implications for the evolution of male courtship displays

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Understanding diversity has been a pursuit in evolutionary biology since its inception. A challenge arises when sexual selection has played a role in diversification. Questions of what constitutes a 'species', homoplasy vs. synapomorphy, and whether sexually selected traits show phylogenetic signal have hampered work on many systems. Peacock spiders are famous for sexually selected male courtship dances and peacock-like abdominal ornamentation. This lineage of jumping spiders currently includes over 90 species classified into two genera, *Maratus* and *Saratus*. Most *Maratus* species have been placed into groups based on secondary sexual characters, but evolutionary relationships remain unresolved. Here we assess relationships in peacock spiders using phylogenomic data (ultraconserved elements and RAD-sequencing). Analyses reveal that *Maratus* and the related genus *Saitis* are paraphyletic. Many, but not all, morphological groups within a 'core *Maratus*' clade are recovered as genetic clades but we find evidence for undocumented speciation. Based on original observations of male courtship, our comparative analyses suggest that courtship behaviour and peacock-like abdominal ornamentation have evolved sequentially, with some traits inherited from ancestors and others evolving repeatedly and independently from 'simple' forms. Our results have important implications for the taxonomy of these spiders, and provide a much-needed evolutionary framework for comparative studies of the evolution of sexual signal characters.

ADDITIONAL KEYWORDS: Australia – character evolution – multimodal signals – rapid evolution – sexual selection – taxonomy – ultraconserved elements.

INTRODUCTION

Sexual selection has driven the evolution of a spectacular diversity of colours, sounds, smells, shapes and forms across the animal kingdom, from darters (Hulse *et al.*, 2020) to bower birds (Frith & Frith, 2004) to mantis shrimps (Porter *et al.*, 2010) to treehoppers (Rodriguez *et al.*, 2004). One common theme that has

emerged from many of these studies is that sexually selected traits sometimes show low phylogenetic signal (Kusmierski *et al.*, 1997; Gleason & Ritchie, 1998; Ohmer *et al.*, 2009; Puniamoorthy *et al.*, 2009; Hosner & Moyle, 2012; Hebets *et al.*, 2013; Owens *et al.*, 2020), often in the traits that are the most obvious to human observers (i.e. bower bird plumage and bowers, hind wings in swallowtails, tyrant flycatcher plumage, frog calls, etc.). It thus follows that for comparative studies, particularly those focused on the evolution

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of potentially homoplastic sexually selected traits, species hypotheses and phylogenetic relationships should be based on independent evidence (e.g. phylogenomic data).

Peacock spiders of the genus *Maratus* Karsch 1878 comprise a diverse clade of jumping spiders (Fig. 1; Supporting Information, Fig. S1) distributed predominately in eastern and western Australia, with over 90 described species (Otto & Hill, 2019a; World Spider Catalog, 2020) and active on-going species discovery (e.g. Schubert & Whyte, 2019; Schubert, 2020). Sexual dimorphism is profound in this group, with cryptic adult females contrasting with highly decorated adult males. Male *Maratus* are now famous for their elaborate visual and vibrational courtship displays (Girard *et al.*, 2011; Otto & Hill, 2011; Girard & Endler, 2014; Girard *et al.*, 2018; Otto & Hill, 2019a; Wilts *et al.*, 2020), facilitated by conspicuous behaviour in which they raise their often colourful abdomens vertically and along with elongated, brush-adorned, third legs perform a vigorous courtship display. Some male peacock spiders have cuticular flaps that wrap



Figure 1. Peacock spiders in courtship posture and variation in fan morphology. A, *Maratus sarahae* – elliptical fan. B, *M. calcitrans* – no fan. C, *M. mungaich* – elliptical fan. D, *M. pardus* – elliptical fan. E, *M. amabilis* – elliptical fan. F, *M. vespa* –posterior lobed fan. G, *Saratus hesperus* – no fan. H, *M. flavus* – no fan. I, *M. rainbowi* – round fan. J, *M. plumosus* – posterior lobed fan. K, *M. harrisi* – lobed fan. L, *M. anomalus* – no fan. M, *M. jactatus* – round fan. N, *M. volans* – elliptical fan. O, *M. linnaei* – no fan. P, *M. clupeatus* – elliptical fan. Q, *M. cinerus* – no fan. R, *M. personatus* – no fan. S, *M. elephans* – elliptical fan. T, *M. madelineae* – elliptical fan. U, *M. proszynskii* – no fan. V, *M. australis* – lobed fan. W, *M. spicatus* – no fan. X, *M. vespertilio* – lobed fan. Y, *M. pavonis* – round fan. Z, *M. speciosus* – no fan. Latitude and longitude data for all specimens can be found in Supporting Information, Table S1.

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beneath the abdomen (Dunn, 1947; Waldock, 1993; Hill, 2009), unfurled during the male courtship display, revealing remarkable patterns of scale pigmentation and structural colours (Stavenga *et al.*, 2016; Hsiung *et al.*, 2017, 2019; Wilts *et al.*, 2020; Fig. 1; Fig. S1; Table S1). The extensive radiation of peacock spider courtship ornaments and displays is perhaps comparable to that of the better-known birds of paradise (Cooper & Forshaw, 1977; Nunn & Cracraft, 1996; Ligon *et al.*, 2018), but these spiders have remained obscure until recently because of their small size. Despite tremendous sexual signal diversity seen in this group, the evolutionary patterns and processes of ornament evolution remain mostly unstudied due to a lack of formal phylogenetic study and resolution.

Previous molecular systematics research has placed Maratus within a clade of mostly Australasian salticids in the Saitis Clade (Fig. 2A; Zhang & Maddison, 2013), within the tribe Euophrvini (Maddison, 2015; Zhang & Maddison, 2015). Zhang & Maddison (2015) considered the multiple genera in the Saitis Clade (Maratus, Saratus Otto & Hill 2017a, Hypoblemum Peckham & Peckham 1885, Jotus Koch 1881, Saitis Simon 1876, etc.) as so closely related as to be possibly congeneric. Conversely, other authors have maintained the above taxa as separate genera (Otto & Hill, 2012a, 2019a; Otto & Hill, 2017a; Prószyński et al., 2018; Baehr et al., 2019). A hypothesized sub-lineage in this clade includes the 'Maratus group' (Otto & Hill, 2012a, 2019a; Otto et al., 2019), including Maratus, Saratus, 'Lycidas' (now mostly synonymized with Maratus) and Hypoblemum. Otto et al. (2019) suggested that the two species of *Hypoblemum* are sister to peacock spiders, including Maratus and Saratus (Fig. 2B). Saratus is monotypic, with male and female genitalia that differ from *Maratus*, but with somatic morphology, male coloration and male display behaviours otherwise similar to the latter (Otto & Hill, 2017a).

Except for the Sanger-sequencing-based studies of Zhang & Maddison (2013, 2015), relationships within the Saitis Clade have never been tested via phylogenetic analysis, although authors have inferred relationships based on character argumentation (Fig. 2B). A unique aspect of generic-level diagnoses in this clade is that many are based mostly or entirely on male courtship morphology and behaviour (Otto & Hill, 2012a, 2017a; Prószyński et al., 2018; Baehr et al., 2019; Otto et al., 2019), rather than the traditional (for salticid systematics) male and/or female genitalic morphology (see Zhang & Maddison, 2015). It remains to be seen whether independent evidence (e.g. molecular evidence) supports taxa based on male courtship characters, or rather, if such characters might be subject to higher levels of homoplasy, calling into question current generic-level diagnostic traits (see also Zhang & Maddison, 2015).

The genus Maratus included fewer than ten described species in 2008 (Waldock, 2008). The description of new Maratus species has exploded over recent years, as interest in these spiders has increased, and macrophotography and video of live specimens has facilitated the discovery of complex male courtship ornaments and behaviours (Otto & Hill, 2019a; Schubert, 2020). Most peacock spider species have been placed into complexes of related species (species groups) based on male morphology and/or display characters (Otto & Hill, 2019a). As of early 2020, 16 species groups have been recognized (Table 1). Relationships within and among groups have not been independently tested using other types of evidence, and resolution within these groups is mostly lacking. Some species are not currently placed into species groups (Table 1) and it remains unclear whether these taxa are truly phylogenetically isolated, or are nested cryptically within described groups. Also, some species appear to share morphological features of multiple groups (Schubert, 2020), blurring species group limits. Similar to arguments above for generic-level diagnoses and because species groups are defined mostly on male courtship morphologies and behaviours (but see Baehr & Whyte, 2016), current phylogenetic hypotheses do not present an independent framework to understand potential homoplasy in these same character systems (Maddison & Hedin, 2003).

Of the now more than 90 accepted species in Maratus, no species hypotheses have been independently tested using molecular evidence. There are many species known only from single (type) locations, and these could represent divergent populations of otherwise known species (i.e. geographical variation). Some 'varieties' have been described within wide-ranging species [e.g. M. pavonis (Dunn 1947); Hill & Otto, 2011; Otto & Hill, 2012a; Baehr & Whyte, 2016], possibly indicating undetected speciation. Some of these wide-ranging species have conspicuously disjunct distributions with populations along the eastern seaboard and in south-western Australia, but populations are absent from the more xeric temperate arid zone [e.g. eastern vs. western *M. pavonis*, *M. vespertilio* (Simon 1901)]. Finally, researchers have discussed possible evidence for gene flow across species boundaries (Otto & Hill, 2014b, 2016a; Schubert, 2020), again blurring species limits, and testable using genetic data.

We collected ultraconserved element (UCE) and RAD-sequencing (RAD-seq) phylogenomic data to address relationships in the *Saitis* Clade, emphasizing *Maratus* relationships. Our sample includes type species for core genera [*Jotus auripes* L. Koch 1881, *Saitis barbipes* (Simon 1868), *Hypoblemum griseum* (Keyserling 1882), 'Lycidas' anomalus (=Maratus anomalus) (Karsch 1878), Saratus hesperus Otto & Hill 2017a, and Maratus amabilis (Karsch 1878)]. For



B) Compilation - multiple sources, see legend



Figure 2. Overview of the phylogenetic structure of the *Saitis* clade. A, results of Zhang & Maddison (2013: fig. 1), based on maximum-likelihood analysis of four concatenated genes. Annotation of uncertain taxon names reflects UCE by-catch phylogenetic results (see Supporting Information, Fig. S3). B, hypothesized phylogenetic structure of the *Saitis* Clade from multiple sources (Richardson & Żabka, 2007; Otto & Hill, 2012a, 2016a, 2017a; Prószyński *et al.*, 2018; Otto *et al.*, 2019; Baehr *et al.*, 2019), with partial list of supporting characters for genera and generic groupings.

Groups (no. of species)	Otto & Hill (2019a) characters; see also Baehr & Whyte (2016)	Otto & Hill (2019a) species (taxa described after 2019 denoted as such)	Taxa sampled in this study	RAD results
fimbriatus group (4)	Wheel-rim-shaped embolus; legs I extended in courtship	 M. fimbriatus Otto & Hill 2016a, M. licunxini Baehr & Whyte 2016, M. speculifer (Simon 1909), M. volpei Schubert 2020 	M. speculifer, M. flame', M. 'carmel'	
chrysomelas group (3)	Apex of the embolus with con- vergent inner and outer edge	M. chrysomelas (Simon 1909), M. kiwirrkurra Baehr & Whyte 2016, M. nigromaculatus (Keyserling 1883)	M. chrysomelas, M. nigromaculatus	
spicatus group (4)	Conical proximal tegulum, etc.; probably related to <i>chrysomelas</i> group	M. nimbus Otto & Hill 2017b, M. purcellae Otto & Hill 2013a, M. robinsoni Otto & Hill 2012a, M. spicatus Otto & Hill 2012a	M. nimbus, M. purcellae, M. robinsoni, M. spicatus	<i>spicatus</i> group intermixed with <i>chrysomelas</i> group
<i>pavonis</i> group (8)	Target on fan, sometimes ob- scured; zigzag ridges on em- bolic disc	 M. leo Otto & Hill 2014b, M. literatus Otto & Hill 2014b, M. maritimus Otto & Hill 2014b, M. montanus Otto & Hill 2014b, M. pavonis (Dunn 1947), M. rainbowi (Roewer 1951), M. sylvestris Otto & Hill 2019c, M. waatagansi Otto & Hill 2013b 	M. cf. leo, M. leo, M. literatus, M. maritimus, M. pavonis, M. rainbowi, M. watagansi	
anomalus group (11)	Bifurcated apex of the outer ring of embolus	 M. albus Otto & Hill 2016b, M. anomalus (Karsch 1878), M. aurantius Otto & Hill 2017a, M. cinereus Otto & Hill 2017a, M. inaquosus Schubert 2020, M. julianneae Baehr & Whyte 2016, M. kochi (Zabka 1987), M. lentus Otto & Hill 2017a, M. vultus M. meptunus Otto & Hill 2017a, M. vultus Otto & Hill 2016b 	M. albus, M. anomalus, M. aurantius, M. cinereus, M. michaelorum, M. neptunus, M. cf. neptunus, M. vultus	Includes <i>M. sceletus</i> from calcitrans group
vespertilio group (2)		M. sapphirus Otto & Hill 2017b, M. vespertilio (Simon 1901)	M. vespertilio	
velutinus group (2)	Squamous setae on abdomen	M. proszynskii Waldock 2015, M. velutinus Otto & Hill 2012a	M. proszynskii, M. velutinus	
<i>harrisi</i> group (3)	Lobate flaps on fan	M. constellatus Schubert 2020, M. harrisi Otto & Hill 2011, M. lobatus Otto & Hill 2016b	M. harrisi	
calcitrans group (7)	Males with asymmetric dis- play, most inflating spin- nerets	 M. calcitrans Otto & Hill 2012b, M. digitatus Otto & Hill 2012b, M. eliasi Baehr & Whyte 2016, M. jactatus Otto & Hill 2015a, M. ottoi Baehr & Whyte 2016, M. plumosus Otto & Hill 2013b, M. sceletus Otto & Hill 2015a 	M. calcitrans, M. digitatus, M. eliasi, M. jactatus, M. ottoi, M. plumosus, M. cf. plumosus, M. sceletus	M. plumosus, M. cf. plumosus near tasmanicus group, M. sceletus in anomalus group
volans group (3)	Large fringed fans with dis- tinctive figures	M. elephans Otto & Hill 2015b, M. pardus Otto & Hill 2014c, M. volans (O. Pickard- Cambridge 1874)	M. elephans, M. pardus, M. volans	M. pardus in expanded mungaich (*from south-west Australia)

Table 1. Continued				
Groups (no. of species)	Otto & Hill (2019a) characters; see also Baehr & Whyte (2016)	Otto & Hill (2019a) species (taxa described after 2019 denoted as such)	Taxa sampled in this study	RAD results
tasmanicus group (3)	Triangular fan with lobes, black spots	M. australis Otto & Hill 2016b, M occasus Schubert 2019b, M. tasmanicus Otto & Hill 2013b	M. australis, M. tasmanicus	M. australis in expanded mungaich (*from south-west Australia)
mungaich group (10)	Wide fans with red scales; south-west Australia	 M. avibus Otto & Hill 2014a, M. bubo Otto & Hill 2016b, M. caeruleus Waldock 2013, M. gemmifer Otto & Hill 2017c, M. hortorum Waldock 2014, M. karrie Waldock 2013, M. madelineae Waldock 2014, M. melindae Waldock 2013, M. mungaich Waldock 1995, M. sarahae Waldock 2013 	M. avibus, M. caeruleus, M. madelineae, M. mungaich, M. sarahae	Not monophyletic, part of ex- panded <i>mungaich</i>
flavus group (4)	south-west Australia endemic, related to <i>linnaei</i> and <i>vespa</i> groups?	 M. boranup Otto & Hill 2018a, M. felinus Schubert 2019, M. flavus Otto & Hill 2018a, M. suae Schubert 2020 	M. flavus	Part of expanded <i>mungaich</i>
linnaei group (4)	Rotating abdomen during courtship; south-west Aus- tralia; related to vespa group?	M. cuspis Otto & Hill 2019c, M. electricus Otto & Hill 2017c, M. laurenae Schubert 2020, M. linnaei Waldock 2008	M. linnaei	Part of expanded mungaich
personatus group (2)	south-west Australia	M. banyowla Otto & Hill 2019b, M. personatus Otto & Hill 2015c	M. personatus	Part of expanded mungaich
vespa group (10)	Lobate flaps with lateral orna- ments; south-west Australia, related to <i>linnaei</i> group?	 M. aquilus Schubert 2019, M. azureus Schubert 2020, M. combustus Schubert 2019, M. cristatus Otto & Hill 2017c, M. icarus Otto & Hill 2019c, M. noggerup Schubert 2020, M. tortus Otto & Hill 2018b, M. unicup Otto & Hill 2018b, M. vespa Otto & Hill 2016b 	M. vespa	Part of expanded mungaich
Taxa not placed into spe- cies groups (6)		 M. amabilis (Karsch 1878), M. clupeatus Otto & Hill 2014d, M. sagittus Schubert & Whyte 2019, M. speciosus (O. Pickard-Cambridge, 1874), M. tessellatus (south-west Australia) Otto & Hill 2016b, M. trigonus (south-west Australia) Otto & Hill 2017c 	M. amabilis, M. clupeatus, M. speciosus	<i>M. clupeatus</i> in ex- panded <i>mugaich</i> (*from south-west Australia)

peacock spiders (*Maratus* and *Saratus*), we generated data for a representative sample of species, including species from all 16 currently recognized species groups (Table 1). Our phylogenomic results reveal that both *Saitis* and *Maratus*, as currently recognized, are paraphyletic taxa. Most described species are recovered as exclusive genetic groupings, but we find evidence for undetected speciation and we present data for three probable new species. Phylogenetic comparative analysis of male visual and vibratory signals reveals a combination of synapomorphic and homoplastic signal. Overall, our research establishes a phylogenetic framework for future studies of peacock spider morphology, behaviour and evolution.

MATERIALS AND METHODS

For a majority of specimens, genomic DNA was extracted from legs or whole spiders using a QIAamp DNA mini kit (Qiagen, Valencia, CA, USA). *Saitis barbipes* samples used in UCE experiments were extracted using a Qiagen DNeasy blood and tissue kit.

TAXONOMIC CORRECTION

The name Maratus rainbowi (Roewer, 1951) is used in this study, in accordance with the World Spider Catalogue (2020), replacing the use of Maratus splendens common in other catalogues (Otto & Hill, 2019a). The name Attus splendens was assigned to a North American salticid species by Peckham & Peckham (1883), which is now considered to be a junior synonym of Habronattus decorus (Blackwall, 1846). Rainbow (1896) also established a species that he called Attus splendens for a new species from Australia, overlooking the prior usage by Peckham & Peckham (1883). Attus splendens Rainbow, 1896 was transferred to Saitis by Simon (1901) and to Maratus by Żabka (1991). In the meantime, Roewer (1951) recognized that Attus splendens Peckham & Peckham, 1883 and Attus splendens Rainbow, 1896 were primary homonyms, and provided the replacement name Saitis rainbowi Roewer, 1951. With the transfer of all Australian species of Saitis to Maratus, this species is correctly known as *Maratus rainbowi* (Roewer, 1951). This usage is in accordance with the International Code of Zoological Nomenclature (1999).

UCE DATA

Specimens of Jotus auripes, Saitis barbipes, Saitis mutans Otto & Hill 2012a, Saitis virgatus Otto & Hill 2012a, Hypoblemum griseum, H. scutulatum L. Koch 1881, Saratus hesperus, and a subsample of seven Maratus species were included in UCE experiments

(Supporting Information, Table S1). We also included the more divergent genus Servaea Simon 1888 to root phylogenies (data shared by W. Maddison), following the results of Zhang & Maddison (2013, 2015). We lacked samples of Barraina Richardson 2013, Maileus Peckham & Peckham 1907 and Prostheclina Keyserling 1882, all hypothesized members of the Saitis Clade (Zhang & Maddison (2013, 2015). We used the MYbaits Spider v.1 kit (Arbor Biosciences, Ann Arbor, MI, USA; Kulkarni et al., 2020) to capture UCE loci, using methods of library preparation as in recent publications (Starrett et al., 2017; Hedin et al., 2019). Sequencing was conducted on an Illumina HiSeq400 at the U.C. Davis Genome Center with 150-bp paired-end reads. Sequence reads were trimmed and assembled using TRINITY v.2.1.1. (Grabherr et al., 2011) using default settings (trimmomatic = full_cleanup, kmer = 25), then processed using PHYLUCE (Faircloth, 2016). Assembled contigs were matched to probes using standard minimum coverage and minimum identity values (80_80). UCE loci were aligned with MAFFT (Katoh & Standley, 2013) and trimmed with GBLOCKS (Castresana, 2000; Talavera & Castresana, 2007) using strict settings (--b1 0.5 --b2 0.85 --b3 4 --b4 8). Resulting PHYLUCE alignments with at least 70% sample occupancy were imported into Geneious 11.0.4 (Biomatters, Auckland, New Zealand), where all individual alignments were visually inspected.

Maximum-likelihood (ML) phylogenetic analyses were conducted using IQ-TREE v.2.0-rc2 (Nguyen et al., 2015). Initial partitions corresponded to individual loci, and ModelFinder (Kalyaanamoorthy et al., 2017) was then used to find best-fit models and merge partitions (-s -p -m TESTMERGE -rcluster 10); the relaxed hierarchical clustering algorithm (Lanfear et al., 2014) was used to reduce computational burden. Support was assessed via 1000 ultrafast bootstrap replicates (Hoang et al., 2018). An SVDQuartets analysis (Chifman & Kubatko, 2014; Chifman & Kubatko, 2015) was conducted on a concatenated matrix using PAUP* 4.0a (Swofford, 1988), implementing the multispecies coalescent tree model with exhaustive quartets sampling and 1000 bootstrap replicates.

Using IQ-TREE 2 we calculated gene (gCF) and site concordance (sCF) factors. For every branch of a reference tree, gCF can be defined as the percentage of 'decisive' gene trees containing that branch, while sCF can be defined as the percentage of decisive sites (in an alignment) supporting a branch (Minh *et al.*, 2020). The latter support metric is particularly useful when individual gene trees are uncertain. Because ML and SVDQuartets UCE tree topologies agreed (see below), we used the partitioned ML tree as a reference for CF calculations, with individual gene trees calculated as default in IQ-TREE 2 (-S command).

UCE BY-CATCH

We assembled ribosomal 28S and mitochondrial 16S_ND1 'by-catch' using standard BLAST searches in Geneious 11.0.4 (Biomatters). Specifically, we used published sequences to query UCE TRINITY assemblies using blastn (maximum e-value of 1e-5). We then stitched together UCE by-catch data with published sequences from Zhang & Maddison (2013, 2015), aligned matrices manually, and conducted ML analyses using IQ-TREE 2 (-s, -B 1000, -bnni). Our primary interest here was to potentially resolve uncertain taxon identities from Zhang & Maddison (2013) (e.g. see Fig. 2A), while also including *Maileus* and *Prostheclina*, not sampled in other matrices.

RAD DATA COLLECTION

Specimens of Jotus auripes, Saitis mutans, S. virgatus, Hypoblemum griseum, H. scutulatum and Saratus hesperus were included as 'outgroup taxa' (Supporting Information, Table S1). Lacking Servaea RAD data, we used UCE topology results to root RAD trees. Specimens representing 48 described Maratus species were included, based on 2013-2015 collections from eastern and western Australia (ACT, NSW, QLD, SA, TAS, VIC, WA; Table 1; Appendix S1). One to three individuals per species were sampled per location, and for more broadly distributed species we attempted to sample populations spanning known geographical ranges. Our taxon sample included five novel forms (Maratus cf. neptunus, M. 'carmel', M. 'flame', M. cf. *leo*, *M*. cf. *plumosus*), representing potentially new species.

We generated RAD libraries using the protocol of Ali *et al.* (2016) without completing the targeted bait capture step and using Pippin Prep (Sage Science, Beverley, MA, USA) instead of beads to size-select fragments between 250 and 600 bp. Each of two 96-sample libraries was sequenced on an Illumina HiSeq 4000 lane at the U.C. Davis Genome Center with 150-bp paired-end reads. Raw fastq reads were de-multiplexed allowing one barcode mismatch. De-multiplexed reads were removed if the expected cut site (also one mismatch allowed) was not found at the beginning of the 5'-end of sequences.

Two separate sets of analytical pipelines and downstream analyses were conducted on RAD data: (1) a custom pipeline was used to extract a set of loci conserved across all taxa – this locus set was used to reconstruct a phylogeny for *Maratus* and 'outgroups'; (2) based on the 'all taxa' RAD results, the ipyrad pipeline (Eaton *et al.*, 2017) was used to assemble locus sets for well-supported species groups (or clades comprising multiple species groups). Because ipyrad analyses were focused on more closely related sets of taxa (rather than all taxa), this enabled us to extract more loci per clade.

RAD ANALYSIS - ALL TAXA

A custom script was used to process RAD data (available at https://github.com/CGRL-QB3-UCBerkeley/RAD), calling various external programs. This pipeline has been used in several other recent publications (Krohn et al., 2018; Maas et al., 2018; Klonoski et al., 2019). Exact duplicates were removed using Super Deduper (https://github.com/dstreett/Super-Deduper). Raw reads were filtered using Cutadapt (Martin 2011) and Trimmomatic (Bolger et al., 2014) to trim adapter contaminations and low-quality reads. The resulting cleaned forward reads of each individual were first clustered using Cd-hit (Li & Godzik, 2006; Fu et al., 2012), and only clusters with at least two reads supported were kept. To remove potential paralogues, Blastn (Altschul et al., 1990) was used to compare clustered loci against themselves, and remove any locus that matched to a locus other than itself. The resulting RAD loci from each individual were then combined for all individuals and the resulting marker sets served as a master reference. We then used Blastn (Altschul et al., 1990) to compare markers from each individual to the master reference and only kept those that had unique hits. These uniquely matched markers from each individual served as a reference for that individual. Cleaned sequence reads from each individual were aligned to its own reference using Novoalign (http://www.novocraft.com) and reads that mapped uniquely to the reference were kept. Picard (http://picard.sourceforge.net) was used to add read groups and GATK (McKenna et al., 2010) to perform realignment around indels. SAMtools/BCFtools and 'vcfutils.pl vcf2fq' implemented in SAMTools (Li et al., 2009) were used to generate individual consensus sequences by calling genotypes and incorporating ambiguous sites. We kept a consensus base only when its depth was at least 3× or above and retained loci that contained no more than 80% missing data. We also masked sites within 5-bp windows around indels. We converted the resulting consensus fastq sequence file to fasta format using Seqtk (https://github.com/ lh3/seqtk). Using MAFFT (Katoh & Standley, 2013), final filtered loci from each individual were aligned by comparing against the master reference. Ambiguously aligned regions were then trimmed using Trimal (Capella-Gutierrez et al., 2009). To avoid excess missing data, we removed alignments if more than 90% missing data were present in at least 30% of samples.

Using a subsample of the entire available sample (152 specimens), we performed several all-taxa phylogenomic analyses. Species trees were inferred

S2. Character scorings for Saitis barbipes were

extracted from other sources (Hill, 2009; https://

using the multispecies coalescent model implemented in SVDQuartets. Originally developed for unlinked single nucleotide polymorphism (SNP) data, where each site has an independent genealogy drawn from a coalescent model, the method has also been shown to perform well for linked SNP data (Chifman & Kubatko, 2014), as used here. SVDQuartets was used to construct a 'lineage' tree, relating individual sequences. We also used a taxon partition to assign individual samples to species (partition = species); for *M. pavonis*, which includes multiple well-supported sub-lineages (see Results below), each sub-lineage here was treated as a 'species'. For both lineage and partitioned analyses we evaluated all possible guartets (evalg = all, quartet assembly algorithm = QFM),and conducted a non-parametric bootstrap analysis, resampling with replacement both loci and sites within loci (bootstrap = multilocus, loci = combined, nreps = 100). IQ-TREE was also used to reconstruct an ML tree from a concatenated matrix, with a single best-fit model automatically chosen by ModelFinder, and support assessed via 1000 ultrafast bootstrap replicates (-s, -B 1000, -bnni).

RAD ANALYSIS - SPECIES GROUPS

Based on RAD phylogenomic results from alltaxa analyses (see below), additional analyses were conducted for members of four wellsupported clades (FC = fimbriatus + chrysomelas, pavonis, anomalus, MTVC = expanded*mungaich* + *tasmanicus* + *volans* + *calcitrans*). Here, forward read (R1) RAD data were de novo assembled using ipyrad v.0.7.30 (Eaton & Overcast, 2016), with the following settings adjusted from default: max Indels_locus = 4, clust_threshold = 0.85 (within and across), min_samples_locus = 5; see Eaton et al., 2017). We used a phylogenetic invariants analysis (Lake, 1987; Chifman & Kubatko, 2015) on linked SNPs to infer quartet trees and a species tree using tetrad v.0.7.30, part of the ipyrad.analysis toolkit, sampling all quartets with 100 bootstrap replicates.

COURTSHIP CHARACTERS

Adult male visual and vibrational courtship signals were characterized using video and laser vibrometer recordings (see Girard *et al.*, 2011 for a detailed description of methods). From these recordings we scored nine characters for all taxa, based on original observations: (1) lateral fan flaps, (2) overall fan shape, (3) raises fan, (4) third leg use, (5) white brushes, (6) elongated spinneret display, (7) vibratory display, (8) vigorous tapping and (9) pre-mount display. Detailed character descriptions and scorings can be found in Supporting Information, Appendix S1 and Table

www.youtube.com/watch?v=wQT2bHTOdwo); some scorings for *Hypoblemum scutulatum* were taken from the courtship description of Otto *et al.* (2019). The characters presented here are not intended to capture the full diversity or complexity of courtship characters seen within *Maratus*, but rather represent a set of scoreable traits with hypothesized deeper homology across all genera included.

CHARACTER EVOLUTION

For tree-based character evolution analyses we used pruned UCE and all-taxa RAD matrices, removing duplicate specimens/populations for individual species, while retaining the most data-rich samples (summarized using AMAS; Borowiec, 2016). We then conducted partitioned and unpartitioned ML analyses of these matrices, as outlined above. Mesquite v.3.6 (build 917) (Maddison & Maddison, 2018) was used to reconstruct ancestral states for courtship phenotypes, scored as alternative discrete states (Supporting Information, Table S3). ML character reconstructions were conducted using the one-parameter Markov k-state model (Lewis, 2001).

RESULTS

Voucher specimen data and relevant summary values are presented in Supporting Information, Table S1. Raw UCE and RAD data have been submitted to the Short Read Archive (BioProjects PRJNA667490 and PRJNA665271), and all data matrices and resulting tree files referenced below are available at Dryad (https://doi.org/10.5061/dryad.9p8cz8wdp).

UCE DATA

The final primary 70% occupancy UCE matrix included 472 loci, with a combined alignment length of 282 674 bp and 39 224 parsimony-informative sites. ML and SVDquartets tree topologies were identical, and both were well supported, with almost all nodes receiving maximum support (Fig. 3; Supporting Information, Fig. S2). Both gene (gCF) and site concordance (sCF) factors were relatively high, except for a node uniting *Saitis mutans* with 'Maratus group' genera.

The genus Saitis is not recovered as monophyletic (Fig. 3). Saitis virgatus is sister to Jotus auripes, possibly misplaced in Saitis. Saitis mutans and Saitis barbipes are also not recovered together; instead they are found to be successive sister species to 'Maratus group' taxa (Hypoblemum, Maratus, Saratus; Fig. 2B).



Figure 3. UCE maximum-likelihood phylogeny, with concordance factor (CF) values. All bootstrap values = 100, unless otherwise indicated. Bootstrap values from SVDQuartets analysis are also shown for nodes with values <100.

Maratus is not recovered as monophyletic. One primary branch includes species representing the *fimbriatus* and *chrysomelas* morphological species groups (Table 1), with a conspicuously long branch leading to this clade (Fig. 3). These *Maratus* species are recovered as sister to *Hypoblemum*. We hereafter refer to this entire clade, also recovered in RAD analyses (see below), as the *Hypoblemum* clade. A second primary branch includes other *Maratus* species, with *Saratus* nested within this larger clade. We hereafter refer to this clade as 'core *Maratus*' (also recovered in RAD analyses, see below).

UCE BY-CATCH

Custom Blastn searches returned ND1_16S data from about half of the UCE TRINITY assemblies, and 28S data from almost all assemblies. Most taxa returned approximately full-length 28S sequences (> 7000 bp); these were trimmed to match the Sanger data. Based on phylogenetic placement in gene trees, we tentatively resolve the identity of five samples from Zhang & Maddison (2013, 2015) (see Fig. 2A; Supporting Information, Fig. S2). Two Hypoblemum samples have discordant 28S vs. 16S_ND1 placements; we leave these identified to genus only. Prostheclina appears allied to *Jotus* (e.g. Fig. 2B), with *Maileus* surprisingly nested, although some nodes are poorly supported for these individual gene trees (Supporting Information, Fig. S3).

RAD ANALYSIS - ALL TAXA

Thirty-four samples were removed from downstream analysis because of low raw read counts. Of retained samples, 152 were included in all-taxa phylogenomic analysis. Using the custom pipeline, the number of filtered reads for these samples ranged from 101 077 to 3623 248, with an average coverage of individual RAD loci of 12× (range from 3.0 to 61.1; Supporting Information, Table S1). We recovered between 9400 and 27 893 loci per sample, but the number of shared loci across the entire taxon sample was lower, probably due to the deep divergences among genera. In total we recovered 513 loci (alignment length = 59 997 bp, 11 290 SNPs, 7196 parsimony-informative sites) for the sample including *Jotus*, *Saitis*, *Hypoblemum*, *Saratus* and *Maratus*.

Following UCE results (see above), we rooted phylogenies using *Jotus auripes* + *Saitis virgatus*. With this root placement, neither *Saitis* nor *Maratus* were recovered as monophyletic in unpartitioned ML (Fig. 4; Supporting Information, Fig. S3) or SVDQuartets (Supporting Information, Figs S4, S5) analyses. One primary branch includes members of the *fimbriatus*, *chrysomelas* and *spicatus* morphological species groups as defined by Otto & Hill (2019a; Table 1). As for UCE data, members of this lineage occur on a conspicuously long branch. Members of the *chrysomelas* group are intermixed with species of the *spicatus* group; because the former species were described first, we hereafter refer to this entire larger clade as the *chrysomelas*



Figure 4. RAD all-taxa maximum-likelihood phylogeny. Bootstrap values below 90 are not shown for most nodes. Placement of *Maratus sceletus*, *M. pardus* and *M. plumosus* to the original groups of Otto & Hill (2019a) is designated with arrows.

group (redefined). Many species in the above three groups were previously placed in the genus 'Lycidas' (but not including the type species of 'Lycidas'). Consistent with UCE data, fimbriatus + chrysomelas (FC) clades are recovered as sister to Hypoblemum, and together comprise the Hypoblemum clade. We discuss below possible morphological and behavioural synapomorphies that unite members of this clade.

A second primary Maratus branch includes all other Maratus taxa ('core Maratus'), with Saratus samples nested within this larger clade (Fig. 4; Supporting Information, Figs S4, S5). The 'core Maratus' clade includes the anomalus species group, including both the type species of 'Lycidas', and M. amabilis, the type species of the genus Maratus. Many clades within 'core Maratus' are mostly concordant with the species group divisions of Otto & Hill (2019a), as based on morphological/behavioural similarities. Exceptions include a monophyletic anomalus group, with the inclusion of M. sceletus Otto & Hill 2015a (from the calcitrans group). Maratus plumosus Otto & Hill 2013b, a hypothesized (but divergent) member of the calcitrans group, also falls outside of this group. The tasmanicus group is not recovered as monophyletic, but instead represents a grade sister to the 'expanded mungaich' (EM) clade. The large, poorly resolved EM clade includes species from several groups as follows: M. clupeatus Otto & Hill 2014d (not a straggler), *M. pardus* Otto & Hill 2014c (from the *volans* group), M. australis Otto & Hill 2016b (from the tasmanicus group), and single sampled species from the *flavus*, *linnaei*, *personatus* and *vespa* groups. The EM clade is further discussed below, but this grouping is biogeographically compelling, as all included taxa are endemic to south-west Australia.

RAD ANALYSIS – SPECIES GROUPS

Tetrad results and locus statistics for the FC, *pavonis*, *anomalus* and MTVC clades are shown in Figure 5. Interspecific relationships in tetrad analyses are generally better supported than in ML or SVDQuartets analysis, perhaps reflecting the greater number of loci recovered for each group. An exception is the EM clade – although tetrad analyses included more SNPs and parsimony-informative sites than in all-taxa ML analyses, relationships are conspicuously poorly supported in both (Figs 4, 5).

Almost all *a priori* species are supported as monophyletic on tetrad trees (Fig. 5). One caveat of this claim is that several species with multiple samples only include specimens from the same geographical location. Five species not recovered as monophyletic on ML trees are recovered as exclusive groupings on tetrad trees [*M. chrysomelas* (Simon 1909), *M. leo* Otto & Hill 2014b + *M. cf. leo, M. literatus* Otto & Hill 2014b, M. neptunus Otto & Hill 2017a, M. aurantius Otto & Hill 2017a]. The only clear case of strongly supported non-monophyly involves the geographically widespread *M. pavonis*. In both tetrad and ML results, *M. pavonis* specimens from Western Australia are more closely related to Western Australia M. maritimus Otto & Hill 2014b, with eastern *M. pavonis* populations phylogenetically closer to geographically adjacent *M. literatus* and *M. leo* (Figs 4, 5). This result supports the contention that *M. pavonis* currently includes several distinct species, as supported by both genitalic (Baehr & Whyte, 2016) and male ornamentation characters (Hill & Otto, 2011; Otto & Hill, 2019a). Introgression with geographical neighbours might also be driving this pattern, but if this were true we might expect more lineage non-monophyly (e.g. Western Australia M. pavonis intermixed with Western Australia M. maritimus, etc.).

Multiple analyses support the hypothesis that three novel forms included here represent undescribed species (M. 'carmel', M. 'flame', M. cf. plumosus). Two forms (M. cf. neptunus, M. cf. leo) might best be interpreted as geographical variants (Figs 4, 5), but larger sample sizes and formal integrative species delimitation analyses are needed to rigorously test these species hypotheses.

CHARACTER EVOLUTION

We lacked some character data for Saitis barbipes and *Hypoblemum* (Supporting Information, Table S2), causing ambiguity in certain ancestral character reconstructions. For the sparse UCE taxon sample that importantly includes Saitis barbipes, we reconstruct the common ancestor of the entire Saitis Clade as males using third legs during courtship (character 4, Fig. S6), and with vibrations produced during a premount display (character 8, Fig. S7; see Girard et al., 2011), with reversals observed for both characters. The common ancestor of the Maratus group is unambiguously reconstructed as with males raising their abdomen during courtship (character 1, Fig. S6), consistent with the hypothesis of Otto & Hill (2012a; Fig. 2B). Ancestral state reconstructions for other characters are shown in Figures S6 and S7.

Ancestral state reconstructions for the denser RAD taxon sample that included the full *Maratus* sample (but lacked *Saitis barbipes*) are shown in Supporting Information, Figures S8–S15. These reconstructions suggest the evolution of a strong white brush on the male third leg evolving at the base of 'core *Maratus*' (character 5, Fig. S11). Two characters suggest that the pre-mount display and pre-mount vibrations may be unique in the *Hypoblemum* clade (characters 8 and 9, Figs S14, S15), predicting that missing data for *Hypoblemum* species will conform to the FC clade. The



Figure 5. RAD tetrad results for the FC, pavonis, anomalus and MTVC clades.



Figure 6. Maximum-likelihood ancestral character reconstruction for fan shape, on the skeletal RAD taxon sample phylogeny. Character states are noted on the phylogeny, and are associated with taxon names; all taxa with character state 0 (=no fan) are left unlabelled.

evolution of fan shape diversity across *Maratus* group members is illustrated in Figure 6.

DISCUSSION

Our phylogenomic results reveal that both *Saitis* and *Maratus*, as currently recognized, are paraphyletic taxa. In *Maratus*, some species are more closely related to *Hypoblemum* taxa. A 'core *Maratus*' clade includes several well-supported sub-clades that mostly correspond to morphological species groups, although some hypothesized groupings were not supported. *Saratus* is nested within the 'core *Maratus*' clade. Based on this phylogenomic tree topology, some traits such as using third legs and

the abdomen during courtship are ancestral to the 'core *Maratus*' clade. Other traits, particularly fan shape, show evidence of multiple independent gains and losses throughout what is currently recognized as *Maratus*.

SAITIS CLADE

Zhang & Maddison (2015) define the Saitis Clade as including multiple genera (Saitis, Maratus, Saratus, Jotus, Hypoblemum, Prostheclina, Maileus, Barraina and possibly Margaromma), united in sharing several male genitalic features, including a lamella on the tegular shoulder distinctive for euophryine spiders. Zhang & Maddison (2015) recognized the striking variation in male courtship ornamentation in the

persal (*contra* Otto & Hill, 2012a). during courtship, but to a lesse Information, Fig. S12). The r

clade (Fig. 2B), but viewed genitalia and overall body form as conservative, and suggested that all genera in the clade might represent junior synonyms of *Saitis*. As they suggest, 'the clade is compact enough morphologically that separating ... genera is unnecessary and gives rise to possible paraphyly and uncertain placement of poorly studied species' (30). This perspective was voiced from a comparative vantage that included detailed knowledge of all euophryine spiders, a clade including over 100 genera (Zhang & Maddison, 2015).

A phylogenomic framework for formal revision of the Saitis Clade will require the inclusion of missing genera (Prostheclina, Maileus, Barraina, Margaromma), additional Jotus species and additional Mediterranean Saitis species. The UCE data presented here provide a starting point for such a larger study. While we do not take formal taxonomic action, our results are consistent with the proposal of Zhang & Maddison (2015) that the Saitis Clade is over-split at the generic level. For example, to retain Maratus and Saratus as monophyletic taxa (a criterion we view as fundamental for genera) would require fragmenting Maratus into three additional genera (FC clade, pavonis group, Maratus clade sister to Saratus), with the anomalus clade retaining the name Maratus. Saitis would suffer the same fate. We propose that a more stable and conservative taxonomy would include FC clade members as *Hypoblemum*, with *Saratus* as a junior synonym of Maratus. Saratus was placed in its own genus because its genitalia differ from other described Maratus (Otto & Hill, 2017a), a situation similar to Mediterranean Saitis that are similar in courtship ornamentation but show divergent male pedipalps (Hill, 2009: fig. 3). Both of these examples illustrate that evolution is heterogeneous in the Saitis Clade, with most taxa showing rapid evolution of courtship ornaments with conservative genitalia, but with small clades sometimes reversing this trend (diverging more quickly in genitalia than in courtship ornamentation).

Although again we lack all constituent genera, our phylogenomic results indicate that the Saitis Clade has primary biogeographical roots in Australia. Multiple genera have radiated exclusively or mostly in Australia (Jotus, Maratus, Hypoblemum, Prostheclina, Barraina). The obvious exception is the Mediterranean Saitis barbipes, which we agree probably forms a clade with two other described Mediterranean Saitis (Saitis graecus, Saitis tauricus; Prószyński et al., 2018). This Mediterranean lineage is nested well within the Australian radiation, and phylogenomic depth (Fig. 3) indicates that this biogeographical disjunction is natural, not the result of recent human-mediated dispersal (contra Otto & Hill, 2012a). SPECIES GROUPS

Many phylogenomic clades within Maratus are mostly congruent with the species group divisions of Otto & Hill (2019a), as defined by morphological and/or behavioural characters. In this sense, our results reveal congruence. Baehr & Whyte (2016) propose that details of the male palp, as examined using scanning electron microscopy (SEM), could reveal additional morphological synapomorphies for groups defined mostly by male ornamentation - SEM study of the phylogenomic groups defined herein will be an important next step. We highlight below areas of incongruence between morphology and phylogenomics, particularly as this relates to patterns of morphological and behavioural homoplasy. This includes interrelationships of the species groups themselves, essentially unresolved prior to this study, which reveal further homoplasy.

Although details differ, phylogenomic recovery of the fimbriatus clade, together with the chrysomelas clade (redefined), coincides with the hypotheses of Otto & Hill (2019a, and references therein). Most members of these groups lack typical *Maratus* features, such as tufted legs and a traditional pre-mount display. The pre-mount display (described by Girard et al., 2011) is different in that third legs are not extended at 90° angles from the body, but instead remain touching the substrate. Also, vibrations are not used prior to the modified pre-mount display. Lastly, the use of third legs in male displays is either severely reduced or nonexistent and instead first legs are primarily employed. Exceptions to the above include the nested species M. chrysomelas and M. nigromaculatus (Keyserling, 1883), with standard pre-mount displays, strong white brushes, third leg use, etc. Based on ancestral character reconstructions, these would be independently evolved character states in these taxa (Supporting Information, Figs S10, S11, S15). Further study of the pre-mount display in the two Hypoblemum species will be important to understand whether these behaviours unite a larger *Hypoblemum* clade (FC + *Hypoblemum*); features of the outer embolus ring might also unite these taxa (Baehr & Whyte, 2016; Otto & Hill, 2019a).

Phylogenomics recovers a monophyletic anomalus clade, but with the inclusion of M. sceletus from the calcitrans group. The latter group is defined by courtship traits, including an asymmetric display in which males also display inflated and extended spinnerets (Otto & Hill, 2019a, and references therein). Our results instead show that M. sceletus is allied with a group of grassland species (M. cinereus Otto & Hill 2017a, M. neptunus Otto & Hill 2017a + M. cf. neptunus, M. aurantius) that display spinnerets during courtship, but to a lesser extent (Supporting Information, Fig. S12). The novel phylogenomic placement of *M. sceletus* thus reveals morphological/ behavioural homoplasy at the level of 'core *Maratus*', and a perhaps novel synapomorphy within the *anomalus* clade (Fig. S12).

The poorly resolved EM clade includes species from several groups as follows: *M. clupeatus* (not a straggler), *M. pardus* (from the *volans* group), *M. australis* (from the tasmanicus group), and single sampled species from the *flavus*, *linnaei*, *personatus* and *vespa* groups. Most of these latter groups have been suspected as relatives (Otto & Hill, 2019a, and references therein). Because we only sampled a single species for the latter four groups, we cannot address group monophyly, but all of these groups comprise species restricted to south-west Australia. Moreover, although short-range endemic species with naturally small ranges (Harvey, 2002) are known for several Maratus species groups, all 30 described species in the mungaich, flavus, *linnaei*, *personatus* and *vespa* groups are short-range endemics (Table 1). Distribution records indicate that all species are found in strict allopatry, with very rare and possibly non-existent syntopy (Otto & Hill, 2019a, and references therein; Schubert, 2020). This striking biogeographical pattern also applies to *M. clupeatus*, M. pardus and M. australis, recovered as part of the EM clade (Supporting Information, Fig. S16). Based on the observation that unplaced *M. tessellatus* Otto & Hill 2016b and *M. trigonus* Otto & Hill 2017c are single-site endemics from south-western Australia (Table 1), we predict their ultimate placement in the EM clade.

We thus propose that a clade of at least 35 (described) species exists in the Western Australia global biodiversity hotspot (Rix et al., 2015), all shortrange endemics where allopatry prevails. Despite gathering phylogenomic data with numbers of overall sites and parsimony-informative sites comparable to other species groups, internal relationships within the EM clade are conspicuously unresolved (Figs 4, 5). We hypothesize that an extremely rapid radiation has occurred, and that future phylogenomic studies will confirm and confront this lack of resolution. Otto & Hill (2014a) have discussed rapid evolution for certain members of this complex. Schubert (2020) also suggested that morphological homoplasy between some members of constituent groups [e.g. M. laurenae Schubert 2020 (linneae group) with homoplasy to *mungaich* group] might indicate introgression, again consistent with a rapid radiation (Seehausen, 2004; Meier et al., 2017), and further challenging phylogenetic resolution.

Although members of different subgroups are not recovered within the more inclusive EM clade (e.g. *mungaich* vs. *vespa*), species in these subgroups are clearly different in many respects, and we are not arguing against subgroup monophyly. Instead, it seems that the rate of morphological and behavioural evolution in these subgroups (and the larger EM clade) has out-paced that of the nuclear RAD dataset. A similar dynamic of extremely rapid morphological evolution out-pacing nuclear RAD divergence was found in oasis-dwelling populations of jumping spiders in western North America (Hedin *et al.*, 2020). Regarding the observation of strict allopatry, we hypothesize that this might indicate that described species within distinct subgroups are all part of a single reproductive community, akin to different populations of a single species, with sympatry precluded because of reproductive interference.

Another possibility is that some taxa in the EM clade are misplaced because of nuclear introgression from geographical neighbours, in what are actually more distant phylogenetic relatives [e.g. *M. pardus* (from the *volans* group), *M. australis* (from the *tasmanicus* group)]. If this is the case, introgression has been differential, because not all species sampled from Western Australia fall into the EM clade [*M. specious* (O. Pickard-Cambridge 1874), western *M. pavonis*, *M. maritimus*, etc.].

PATTERNS OF CHARACTER EVOLUTION

In previous behavioural work on *M. volans* (**O**. Pickard-Cambridge 1874), it was demonstrated that visual signals (in particular fan dancing and third leg movements) strongly predicted mate choice (mating success, mating latency, copulation duration) while vibratory signals weakly predicted mate choice (Girard et al., 2015). It follows that some aspects of sexually dimorphic abdominal morphology, third leg morphology and vibratory song play a role in sexual selection and the evolution of the group. In our investigation of courtship character evolution, we focused on the evolution of visual traits (abdominal raising, flaps, fan morphology, third leg tufts) and the presence of vibratory song. The broader pattern of character evolution suggests that some of these key courtship characters are more ancient (use of vibratory songs, third leg use), others evolved in the common ancestor of the Hypoblemum clade and 'core Maratus' (raising of the abdomen), others evolved in the common ancestor of the 'core *Maratus*' group (third leg tufts), and others evolved recently through multiple evolutionary events (lateral fan flaps, fan shape variation).

Abdominal raising allows the presentation of a body part that would otherwise be hidden from view, to be available for assessment in mate choice. The use of the abdomen as a signal is probably important not only because it allows the presentation of patterns important for species recognition (Girard *et al.*, 2018) but also because it can be moved and shaken which could indicate 'quality' (Shamble *et al.*, 2009; Girard et al., 2015). Character reconstruction suggests that the use of the abdomen in courtship evolved twice, possibly independently in the Hypoblemum and Maratus clades, with three losses in Maratus spread amongst the pavonis (M. watagansi Otto & Hill 2013b), anomolus (M. albus Otto & Hill 2016b) and EM clades (M. personatus Otto & Hill 2015c) (Supporting Information, Fig. S8). Losses in abdomen-raising are also associated with losses of abdominal ornamentation. The species involved tend to occupy open sandy habitats (M. albus and M. watagansi), suggesting that this loss may be associated with predator avoidance, although this remains to be tested. The use of the abdomen in signalling has interestingly been demonstrated to be an anti-receptivity signal in female M. volans (Girard et al., 2015) and observations suggest that this type of behaviour may be present in other Maratus (e.g. M. rainbowi, M. plumosus; M. digitatus Otto & Hill, 2012b; M. B. Girard, personal observation; M. digitatus Otto & Hill, 2012b) as well as Maratus ancestors (e.g. Saitis barbipes). These female signals may thus precede the use of abdominal courtship signalling in males.

The use of the abdomen in male courtship has led to the evolution of multiple characters including, but not limited to, flaps and varying shapes, colours and patterns on the abdomen (Figs 1, 6; Supporting Information, Table S2). Our preliminary analyses suggest that abdominal flaps are evolutionarily labile, showing multiple gains and losses (11 and five, respectively) across the phylogeny whether flaps are minimal (e.g. M. pavonis), large (e.g. M. madelineae Waldock 2014) or consisting of elongated bristles (e.g. M. speciosus, M. nigromaculatus; Fig. 6). The overall shape of the fan results from the presence/ extent of modifications to the abdomen. Assumed synapomorphies in fan morphology/shape have been used as characters to distinguish species and species groups and, although mostly concordant, molecular evidence suggests that some fan morphologies have arisen multiple times (see above). Our data support the hypothesis that most fan shapes evolved independently from a common ancestor that did not have a fan. For example, the round fan in several members of the pavonis clade, M. vultus Otto & Hill 2016a from the anomalus clade and M. jactatus Otto & Hill 2015a from the *calcitrans* clade each evolved independently from a 'non-fanned' ancestor. Similar patterns are observed for elliptical (e.g. *M. amabilis*, volans clade, EM clade), lobed (e.g. M. vespertilio, M. harrisi Otto & Hill 2011, M. digitatus, M. australis, M. tasmanicus Otto & Hill 2013b) and posterior-lobed (tasmanicus clade, M. vespa Otto & Hill 2016b) fans. The evolution of complex male morphologies associated with courtship from more simple morphologies is thus common and appears to have arisen through multiple PEACOCK SPIDER PHYLOGENOMICS 17

singular evolutionary events. Finally, our analyses suggest that the EM clade has a wide diversity in fan shapes, suggesting that fan shapes can evolve rapidly (Fig. 6). Alternatively, the diversity of fans in the EM clade may result from adaptive introgression, as some studies have suggested that introgression may be a source of novel morphological traits (reviewed by Abbott *et al.*, 2013; Leduc-Robert & Maddison, 2018).

Our results suggest that displays using the third legs, unlike many of the abdominal traits, are shared with the common ancestor of Saitus, Hypoblemum and Maratus, with some losses in the Hypoblemum clade and in Saratus. Ornamentation on the third legs (tufts) also appears to have evolved independently in the Hypoblemum and 'core Maratus' groups. The ancestor to the 'core Maratus' group probably had third leg tufts with losses in Saratus and the velutinus group (M. velutinus Otto & Hill 2012a and M. proszynskii Waldock 2015). Where examined, vibratory songs have been observed in most of the jumping spider genera that have been studied (e.g. Habronattus, Phidippus, Maevia, Cosmophasis; Gwynne & Dadour, 1985; Maddison & Stratton, 1988; Elias et al., 2005, 2008, 2012, 2014; Uhl & Elias, 2011; Zeng et al., 2019) including Maratus (Girard et al., 2011, 2015, 2018). Our analyses suggest that the common ancestor to all Maratus produced vibratory songs with losses in the FC clade (i.e. M. neptunus, M. aurantinus) and in *M. cinerus*. Vibratory songs are thus ancient and probably important in sexual selection across the entire group and their ancestors.

Spiders have been used to examine the evolution of mating behaviour, particularly jumping spiders in the genus *Habronattus* (Maddison & McMahon, 2000; Masta & Maddison, 2002; Elias *et al.*, 2006; Blackburn & Maddison, 2014; Leduc-Robert & Maddison, 2018) and wolf spiders in the genus *Schizocosa* (Stratton & Uetz, 1981; Miller *et al.*, 1998; Stratton, 2005; Hebets, 2008; Hebets *et al.*, 2013; Rosenthal & Elias, 2019). Similar to the results found in *Maratus*, these studies have found evidence for the accumulation of signalling complexity in some lineages (Elias *et al.*, 2012; Hebets *et al.*, 2013; Herberstein *et al.*, 2014) and repeated reversions to simpler morphologies (Maddison & Hedin, 2003; Elias *et al.*, 2012).

CONCLUSIONS

This study provides a taxonomically broad phylogenetic analysis of peacock spiders using genome-wide markers, the first molecular phylogeny estimated for this group. Our results challenge the current status of peacock spiders as monophyletic and, accordingly, our molecular phylogeny has important implications for the taxonomy of *Maratus* and closely related genera. Our phylogenetic analyses largely corroborate several species groups proposed by Otto & Hill (2019a), while bringing new surprises.

Our data suggest that the evolution of male courtship behaviour in this group has moved toward greater complexity via a number of singular evolutionary events (e.g. use of abdomen in courtship, development of third leg ornamentation, development of courtship vibrations and development of fan flaps). Occasional reversions to simpler morphologies (reduced fan flaps, loss of third leg ornaments or loss of third leg use in courtship displays) have also occurred several times in the course of peacock spider evolution, perhaps most notably in species such as M. personatus, M. velutinus and *M. proszynskii*. There are also specific aspects of courtship complexity that have seemingly emerged independently in different lineages (e.g. elongated and inflated spinnerets). Different fan morphologies also appear to be evolutionarily labile, with multiple evolutionary events stemming from non-fanned ancestors. However, the use of vibratory song in courtship is more ancient, with notable losses in the FC clade.

The role of sexual selection in diversification has been a contentious issue (Rolan-Alvarez & Caballero, 2000; Phillimore et al., 2006; Kraaijeveld et al., 2011; Gomes et al., 2016; Servedio & Boughman, 2017). One take-home message from this literature is that the relationship between sexual selection, diversification, speciation and local adaptation can be extremely nuanced (Servedio & Boughman, 2017). It follows that in systems such as the peacock spiders in which the 'showiness' of male display characters drives assumptions of behavioural and evolutionary patterns, there is a great need to understand speciesand population-level relationships. While much work remains to be done, our study shows that the evolution of displays in the peacock spiders is multifaceted and complex and that there is a need for more systematics research to build the appropriate evolutionary context.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Fig. S1. Additional peacock spiders in courtship posture and variation in fan morphology. (a) *Maratus velutinus* – no fan; (b) *M. amabilis* – elliptical fan; (c) *M. tasmanicus* – elliptical fan; (d) *M. neptunus* – no fan; (e) *M. cf. neptunus* – no fan; (f) *M. sceletus* – no fan; (g) *M. michaelorum* – no fan; (h) *M. digitatus* – lobed fan; (i) *M. robinsoni* – no fan; (j) *M. aurantinus* – no fan; (k) *M. ottoi* – no fan; (l) *M. cf. leo* – no fan; (m) *M. vultus* – round fan; (n) *M. albus* – no fan; (o) *M. leo* – no fan; (p) *M. literatus* – round fan; (q) *M. eliasi* – diamond fan; (r) *M. nigromaculatus* – no fan; (s) 'flame' – no fan; (t) *M. avibus* – elliptical fan; (u) *M. purcellae* – no fan; (v) *M. speculifer* – no fan; (w) *M. chyrsomelas* – no fan; (x) *M. mungaich* – elliptical fan; (y) *M. caeruleus* – elliptical fan. Latitude and longitude data for all specimens can be found in Supporting Information, Table S1.

Fig. S2. UCE SVDQuartets phylogeny.

Fig. S3. ND1_16S and 28S by-catch ML phylogenies.

Fig. S4. RAD all-taxa SVDQuartets species tree, with species partitioning.

Fig. S5. RAD all-taxa SVDQuartets species tree, with lineage partitioning.

Fig. S6. ML ancestral character reconstructions for UCE taxon sample (characters 1, 4 and 5).

Fig. S7. ML ancestral character reconstructions for UCE taxon sample (characters 7–9).

Fig. S8. ML ancestral character reconstruction for RAD taxon sample, character 1-raises abdomen.

Fig. S9. ML ancestral character reconstruction for RAD taxon sample, character 2–lateral fan flap (see main text Figure 6 for character 3 reconstruction).

Fig. S10. ML ancestral character reconstruction for RAD taxon sample, character 4-third leg (leg III) use.

Fig. S11. ML ancestral character reconstruction for RAD taxon sample, character 5–white brush on third leg (leg III) tarsi.

Fig. S12. ML ancestral character reconstruction for RAD taxon sample, character 6–elongated spinnerets display. Fig. S13. ML ancestral character reconstruction for RAD taxon sample, character 7–vigorous tapping display. Fig. S14. ML ancestral character reconstruction for RAD taxon sample, character 8-vibrations produced during pre-mount display.

Fig. S15. ML ancestral character reconstruction for RAD taxon sample, character 9-pre-mount display.

Fig. S16. RAD tetrad phylogeny for the EM clade, with geographical distributions in western Australia. Table S1. Specimen information.

Table S2. Character states for all examined specimens.