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Unravelling the speciation process in barking geckos (*Ptenopus*: Gekkonidae)

by

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Thesis

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DECLARATION

The research reported in this thesis is my original work, completed by me between 2019 and 2023 at the University of Witwatersrand. This work is the result of data collected primarily by me during my registration at Wits, but also largely supplemented by my previous work at Gobabeb Research Station and my current recent work at the National Museum of Namibia, in collaboration with my supervisors and my previous research manager, Eugene Marais. Each contribution to this thesis from the work(s) of other people has been attributed, acknowledged, and referenced as appropriate. I have not allowed, and will not allow anyone to copy my work with the intention of passing it off as his or her own work.



Francois S Becker

31 May 2023

Date

I dedicate this work to my wife, Tessa Becker, who supported my passion for research throughout the years of our marriage, endured my many absences during fieldwork, and picked up the slack at times when this work demanded my full attention. Your warmth, love, and support have been like a guiding light in times of darkness. I dedicate this work to my two children, Abigail and Ian Becker, who have brought more fulfillment to my life than I can describe or could have expected. Your attentions and distractions from my research often lightened my heart and renewed my strength. Furthermore, I am deeply grateful to my parents, Richard and Ebbie Becker, and to my parents-in-law, Simon and Sally van der Lingen, whose help and support I could always count on. Finally, I dedicate this work to my God, in whom I put my trust.

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ABSTRACT

Barking geckos (genus *Ptenopus*) are a group of terrestrial, burrowing lizards endemic to southern Africa, with three currently recognised species: *P. kochi, P. carpi,* and *P. garrulus,* the last containing the subspecies *P. g. garrulus* and *P. g. maculatus*. Males produce uniquely loud vocalisations to attract females to the burrows, a trait which is involved in mate recognition. I produced the first comprehensive phylogeny for the genus, which supported several deep divergences and the presence of unrecognised species. I then applied the unified species concept in an integrated taxonomic framework to delimit species within *Ptenopus.* I used multiple lines of evidence including genetic-spatial analyses, advertisement calls, ecological divergence, and morphology to test the evolutionary independence of putative species suggested by phylogenetic species delimitation analyses. Based on these findings, I suggest there is a total of eight species of *Ptenopus,* and that the two subspecies *P. g. garrulus* and *P. g. maculatus* should be elevated to full species. I provide provisional descriptions of four additional species. The advertisement calls and preferred habitats were clearly distinct among these proposed species, while morphological characters were generally less reliable for species identification.

I also tested various candidate hypotheses of divergence drivers in the genus using multiple regression on matrix models (MRMs). The MRMs supported the hypothesis that substrate specialisation was the main factor over other factors as the key driver of divergence. Geographic barriers, including rivers and mountains or ridges, also appear to be important drivers of some of the more the recent divergences. The Namib Desert is the centre of diversity for the genus *Ptenopus*, containing seven of the eight proposed species. There was also strong evidence for reproductive character displacement in the advertisement calls of sympatric species, indicating the importance of calls in mate recognition and the maintenance of species boundaries. This is the first study to show evidence for reproductive character displacement of vocal traits in a non-avian reptile.

CHAPTER 1 (INTRODUCTION): Integrated taxonomy as a hypothesis-driven discipline

The position that species are the fundamental category of biological organisation (Mayr 1942, Smith 2016) has largely been assimilated into science and society, from the time of Linnaeus (1707-78) until the present day. Darwin proposed a more fluid interpretation of species, proposing that, over time, through the actions of natural selection and repeated reproductive isolation, species are unstable entities that evolve (Darwin 1859). This may seem like an obvious point of view to raise to biologists, but the frequency at which this viewpoint has been misunderstood in the scientific literature with regard to species boundaries, may surprise some. Since the birth of evolutionary theory, there has been much disagreement over what constitutes a species, how it should be defined, and even whether or not it can be considered a 'real' entity, or simply an artificially imposed categorisation. Darwin maintained: "the amount of difference considered necessary to give to any two forms the rank of species cannot be defined"; "… but I shall at least be freed from the vain search for the undiscovered and undiscoverable essence of the term species" (Darwin 1859). Hence, he concludes that species boundaries cannot be, and will never be objectively defined. It might be remarked that biologists have spent well over a century arguing this point, and that some have repeatedly come to the same conclusion (e.g., Stebbins 1969; Joseph and Omland 2009).

The 'unit' of species is the basis for much of the biological sciences, conservation decision-making, international conventions, and nearly all biodiversity-related laws and policies. For a species to be named, a rigorous and quantitative research process is usually required, including several standardised methods and analyses, along with an interpretive component, and a strict adherence to nomenclatural standards as dictated by the International Code of Zoological Nomenclature. However, several authors have claimed that there is unprecedented inconsistency, subjectivity, and even "anarchy" in the taxonomic system (Garnett and Christidis 2017, Holstein and Luebert 2017, Zachos 2018). According to some of these authors, taxonomy is discredited as a science, and the inconsistency of species delimitation causes major problems for scientific analyses, conservation decision-making, and that it is costly to society (Faurby et al. 2016, Garnett and Christidis 2017). Zachos (2018) states, similar to an earlier remark by Darwin: "there is no, and perhaps there never will be, a fully objective way of ranking and delimiting species". He then concludes: "Business as usual is not an option if taxonomy is to be a full-fledged scientific discipline" – thereby implying that it is not.

Garnett and Christidis (2017) even suggested that the decision to name a species should become centralised under the International Union of Biological Sciences (IUBS), to "restrict taxonomic freedom". A string of published criticisms soon followed this proposal, including a paper (Thomson et al. 2018) co-authored by well over a hundred scientists. The main criticisms of Garnett and Christidis' proposal was that taxonomy is a hypothesis-driven science, with evolving methodologies, a developing understanding of what species are, and a constantly improving consensus on the treatment of species (Cotterill et al. 2017, Lambertz 2017, Raposo et al. 2017, Thomson et al. 2018, Taylor et al. 2019). Others have claimed that they misrepresented the adverse effects of taxonomic instability on conservation, and countered with a range of examples showing the opposite to be true (Raposo et al. 2017, Thomson et al. 2018, Taylor et al. 2019). The examples on the instability of species names were also cherry-picked extremes from the more charismatic and less diverse taxonomic groups (large mammals and birds), whereas new species discovery, not repeated reversals, have characterised the more diverse taxa (Thomson et al. 2018). Furthermore, the idea that species simply represent a 'unit' to be used in conservation or analytical applications, rather than the essence of evolution to be studied in the biological sciences, was also criticized and highlighted as a major misunderstanding of taxonomy, and indeed of, species (Jackson et al. 2017). Suggesting that an entire field of science be collapsed to account for another fields' inappropriate choice of model units, is certainly preposterous. However, while the much-contested proposal to bring taxonomic decisions under a socio-political governing body may be misguided and counter-productive (Hollingsworth 2017), the inconsistency of species treatments is worth considering as a potentially real problem.

One of the features of taxonomy that have led to critical views is the wide variety of species concepts that exist. There is even disagreement over the number of species concepts, with reported numbers running up to 20 (Mayden 1997) or 34 (Zachos 2016). Most authors agree that there are three basic 'realms' of species concepts, or even that species concepts can be reduced to the essential three (e.g., see Carstens et al. 2013): the morphological species concept (dating from before the time of Darwin), the genealogical species concept (Baum and Shaw 1995), and the biological species concept (Mayr 1942). Interestingly, the biological species concept had already been extensively reviewed, discussed, and was rejected by both Darwin and Wallace. To quote some conclusive statements from Darwin's 'On the Origin of Species by Means of Natural Selection' (Darwin 1859): "from these several considerations I may conclude that fertility does not constitute a fundamental distinction between varieties and species when crossed" …."neither sterility nor fertility affords any clear distinction between species or varieties"; and from Wallace (1865)"..since theories of speciation involve a reduction in ability or tendency to interbreed, species cannot

themselves be defined by interbreeding without confusing cause and effect." Yet somehow, it still became a textbook definition for many decades after Mayr's (1942) publication, and although criticised by some authors (e.g., Mallet 1995; Hey 2006), it is still one of the most popular species concepts today due to the clear nature of the criteria. One of the major shortcomings of this species concept in a practical sense remains: it is usually impossible to test in an allopatric context (Mayr 1942, Zink and McKitrick 1995).

Turning to Darwin's application of criteria to delineate species, there is no denying that the morphological species concept is also fraught with problems. Most prominently, we now know that processes such as ecological convergence are sure to cause morphological similarities in non-sister, or even distantly related species (McGhee 2011, Tolley et al. 2022). By contrast, sympatric speciation will usually lead to very different morphologies in sister- or closely related species (Rundle and Nosil 2005, Bolnick and Fitzpatrick 2007). The extreme dissimilarity that can result between groups of organisms due to divergent ecological paths is perhaps best exemplified by birds and reptiles, which had been considered separate lineages for centuries before genetic evidence placed birds as a nested clade within reptiles (Hedges 1994, Hedges and Poling 1999).

According to contemporary understanding of evolution (see Huxley 1942, Laland et al. 2015), species and their relationships are to be based on evolutionary trajectories. Hence, a measure of the evolutionary history and relatedness among organisms is necessary to truly test the validity of a species (Mallet 1995). This, in part, is what led to the development of the genealogical species concept (Baum and Shaw 1995), which has become widely accepted. Even in morphology-oriented circles, the inclusion of genetics as a backbone for taxonomic work has become standard (Goldstein and DeSalle 2011, Jörger and Schrödl 2013, Hubert and Hanner 2016). Not only does genetic relationship provide a direct measure of relatedness, but there are simply more features to compare objectively. In any decent morphometric analysis, between 20 – 30 features being measured and compared, would be considered a very thorough analysis (Reutter et al. 1999, Lattig et al. 2007). By contrast, a single mitochondrial gene such as the commonly used cytochrome oxidase subunit I, usually contains >600 objectively comparable features across taxa – and most modern analyses compare multiple genes.

In recent decades, the increased ease and reduced cost of genetic sequencing has resulted in a greatly accelerated rate of species discovery and description (Carstens et al. 2013, Streicher et al. 2020, Womack et al. 2021) leading to something akin to the second 'golden era' of taxonomy. In particular, genetic sequencing has allowed for the identification of vast numbers of cryptic species (Bickford et al. 2007, Streicher et al. 2020, Womack et al. 2021), which occur throughout the tree of life and across the globe (Bickford et al. 2007, Pfenninger and Schwenk 2007). The implication of

these discoveries is that separate lineages often evolve with little or no apparent external morphological changes, but these lineages can still display marked differences in ecology, behaviour, physiology, and their impact on ecosystems (Bickford et al. 2007, Struck et al. 2018).

It has been proposed that phylogenetic methods can be used to standardise species delimitation and taxonomic assignments (Hebert et al. 2003, Hubert and Hanner 2016), and indeed a number of species delimitation analyses exist. Species delimitation analyses have even become a strongly recommended analytical tool for taxonomic publication. These methods normally involve either a threshold value of sequence divergence based on the gap between current intra- and inter-specific divergence, or a tree-based approach (Goldstein and DeSalle 2011, Carstens and Reid 2012, Puillandre et al. 2012, Zhang et al. 2013). However, there has been a trend towards incrementally lower divergence thresholds for classifying genetic clusters as species, or a shift towards the 'splitter' camp. This is often due to a simplified interpretation of genetic divergence, without taking into account various lines of evidence and a spatial interpretation of genetic patterns (Cicero et al. 2021, Tolley et al. 2022). While divergence thresholds can be used as a 'rough guide' to identify cryptic species (Tobias et al. 2010), they can have high failure rates in species identification by themselves (Meier et al. 2006). There are also inconsistencies in the results of different species delimitation analyses, depending on the geographic inclusion of sampling areas (Tolley et al. 2021). To exacerbate these problems, there are various natural processes, such as gene-flow among metapopulations, which can greatly impact the consistency of genetic divergence-based findings (Chan et al. 2022). Most importantly, there are real inconsistencies in the rate of molecular evolution throughout the tree of life, even among relatively closely related species (Bromham 2009, Tobias et al. 2010).

There are even inconsistencies in the rate of evolutionarily significant genetic divergence among closely related species. For example, in an isolation-by-distance model of populations (Wright 1943), two forms can be genetically distant when they represent two extremes along a metapopulation continuum (Telles and Diniz-Filho 2005, Jenkins et al. 2010). By contrast, an ecologically diverged form, even if more closely related to its congeners than in the previous example, can be considered an evolutionarily distinct species (Rundle and Nosil 2005). Another major point to consider is the stability of a divergence event. For instance, two populations may begin to diverge allopatrically, but are later brought back into geographic and genetic contact due to changing environmental conditions (Tolley et al. 2021). Thus, the permanence of a true species-level difference in an allopatric context is more difficult to test, and may require extensive comparisons to closely related sympatric species (Tobias et al. 2010). Closely related forms may remain reproductively isolated due to divergent mate recognition systems despite sympatric occurrence of different forms (e.g., Hobel and Gerhardt 2003), while in other cases, more distantly related forms

may readily interbreed if brought into contact (Zhang et al. 2016). These and other nuances of the speciation process mean that simple rules or categorisations are just not good enough to test whether two forms are truly on evolutionarily separate trajectories. Simply put, species delimitation is 'fuzzy' or 'messy', because natural systems are.

The inconsistency of species treatment in taxonomy is a reflection of the true inconsistency and complexity of natural systems, not (only) a sloppy mishandling of a highly ordered system. However, this complexity does not exclude order from the treatment of species. To this end, there has been a strong movement towards the general lineage species concept (de Queiroz 1998), later reformulated as the unified species concept (de Queiroz 2007). De Queiroz and others (e.g., Hey 2006) argued that species concepts should not be classified by the methods used to delineate species, but rather the theoretical concept underlying our understanding of species and evolution. This concept, consistent with the Modern Synthesis and notably influenced by Mayr, states that species present as metapopulation lineages on different evolutionary trajectories. This species concept has been widely assimilated into the taxonomic literature (e.g., Coetzee et al. 2013; Karin et al. 2016). However, different methodologies still produce different results when it comes to deciding whether to split or lump species, one of the main sources of contention for those wishing greater consistency in the field of taxonomy (Faurby et al. 2016). This is a normal progression in science, with methods being tested, reviewed, and refined as more studies become available on them. Taxonomic science remains highly quantitative and the methods do tend to become more formalised as time progresses and a consensus is reached (Thomson et al. 2018).

The simple application, however standardised, of known methodologies to add or subtract species without due consideration to the relevant system and ecological context, is one of the main pitfalls for taxonomists to avoid (Bickford et al. 2007, Raposo et al. 2017, Thomson et al. 2018). Yet, this seems to be the very approach being advocated by those authors demanding greater consistency in taxonomy. Rather, the framing the species delimitation process as a set of falsifiable hypotheses leads to a scientifically robust species delimitation process. This approach requires aspects of study from biogeography, phylogenetics, and even behavioural ecology. Hence, taxonomy should be treated as an inter-disciplinary science. These aspects of a 'speciation' study are often published separately and are thus seen as disconnected from the actual taxonomic study, with species descriptions being the end-product. However, the understanding of the speciation process is necessary to accurately delineate species boundaries. This inter-disciplinary nature of taxonomy has become the standard for taxonomic papers published in recent decades (Haacke 1975, Bauer et al. 2006, Conradie et al. 2012, 2022, Agarwal et al. 2017, Tolley et al. 2022).

Based on common themes from the literature and some explicit recommendations (Tobias et al. 2010, Dupuis et al. 2012, Pante et al. 2015, Cicero et al. 2021), I suggest several 'hallmarks' of a good speciation study, the end-product of which is a good taxonomic study. The first is a robust sampling design that ensures the relevant hypotheses can be falsified, and that more relevant hypotheses can be generated. This should include sampling across the geographic range of the species or group being assessed, including potentially relevant ecological variation, and including all closely related, and some more distantly related species. The samples to be analysed should include several genes, nuclear and mitochondrial, for an initial phylogenetic analysis, and phenotypic data (morphology, colour, and/or ecologically relevant traits such as calls, breeding or living site selection etc.). Regarding the latter, it is essential to assess which properties of the organisms could be relevant in the speciation process *a priori* – such as traits relevant to reproductive isolation or possible ecological differences – and then quantifying these. Secondly, once the phylogenetic data have been analysed, the spatial connectivity of clades must be explicitly assessed, and potential drivers of speciation identified, which can potentially be further tested. Thirdly, the spatio-temporal stability of these perceived drivers of speciation should be investigated to assess how likely a divergence event would be to maintain species boundaries. Lastly, comparisons should always be made to other taxonomic studies in a similar ecological or taxonomic context, to critically assess whether species delimitation decisions taken in the present study (or even in the compared studies) was done correctly.

Taxonomy is, or should be, a hypothesis-driven discipline that studies the very essence of evolution, not just a procedure for generating a list of extant taxa. This is not always apparent in the way that taxonomic studies are published, and the theoretical background is not always explicitly framed or tested. Integrative taxonomy will continue to produce inconsistent results due to the complex nature of species and speciation. This will continue to be a problem for studies aimed at using species as a consistent 'unit' for quantifying diversity. Various alternative metrics of diversity have been suggested, which may be more appropriate for some applications (e.g., Schmera et al. 2009). However, I consider species to be the most appropriate unit for quantifying the diversity of life, despite, and even because of its inconsistencies. Species are not a quantification of only one type of diversity – genetic, functional, ecological, or aesthetic. Rather, species encompass the balanced summation of the complex set of processes that constitute evolutionary diversity, and the diversity of life.

Study animal: *Ptenopus*

Barking geckos, genus *Ptenopus*, are burrowing geckos with substantial vocal abilities, as the name suggests. Currently, there are three described species: *Ptenopus garrulus* (Smith, 1849), *P. carpi* Brain, 1962 and *P. kochi* Haacke, 1964. The first-described *P. garrulus* or common barking gecko, occurs throughout Namibia, most of Botswana, the northern and south-central portions of South Africa, and the very southern edge of Zimbabwe (Branch 1998). This species has two subspecies, *P. g. garrulus* and *P. g. maculatus* Gray, 1866. The latter subspecies was first described as a species from "Damaraland, South West Africa" (currently Namibia), and was later synonymised with *P. garrulus* by Boulenger (1885). Subsequently, FitzSimons (1935) considered it as a valid subspecies, *P. g. maculatus*. This taxonomic treatment has remained in effect ever since (e.g., Loveridge 1947, Bauer et al. 1993, Branch 1998) despite several revisions of the genus (Brain 1962, Haacke 1964, 1969, 1975), although the geographic boundary between the subspecies had been adjusted several times. *Ptenopus carpi* and *P. kochi* are endemic to the Namib Desert, western Namibia. Koch's barking gecko is restricted to the Namib Sand Sea in the central Namib Desert (see Haacke 1975). Its habitat was originally characterised as the silts and fine sand of the Kuiseb River (Haacke 1964), which is only a very marginal strip of its habitat. Carp's barking gecko or the Namib barking gecko occurs throughout the central and northern Namib Desert plains (Haacke 1975). The central Namib Desert is the centre of diversity for the genus, with all three species occurring here.

All three species can be found in sympatry or parapatry near Gobabeb Research Station, central Namib Desert, which is also the type locality for two species (Brain 1962, Haacke 1964). In this area, occasional flooding of the Kuiseb River blocks the north-eastward movement of the sand dunes. This causes a dramatic change in soil type, with a massive expanse of tall, red sand dunes on the southern side of the river and flat, white-grey gravel plains on the opposite side. *Ptenopus kochi* occurs on the southern side of the river and in the river, while *P. carpi* occurs only on the northern side and *P. garrulus* is found on both sides (own observations, but see: Haacke 1975, 1976). This distribution pattern is probably related to substrate preference, with *P. carpi* occurring on hard, courser-grained gravelly substrates while *P. kochi* prefers soft, loose sand. *Ptenopus garrulus* appears to be a generalist that tolerates both substrates (Brain 1962, Haacke 1964, 1975).

Substrate specificity is probably related to the ground-living lifestyle and burrowing habits of *Ptenopus* (Haacke 1975). All three species dig relatively complex burrows with multiple side-branches and one or more escape exits. These end just beneath the soil surface, allowing the occupant to break through and escape if its burrow is being excavated, or presumably if a predator enters the burrow (Haacke 1975). *Ptenopus kochi* generally has the most, and *P. carpi* the least

elaborate burrow structure (Haacke 1964, 1975), while considerable variation in *P. garrulus* burrow structure has been described from different geographic locations (Haacke 1964, 1975).

Ptenopus have a number of adaptations for burrowing, including fringed scales on the toes and, to a lesser extent, the fingers, which increase the surface area for digging and scooping soil (Brain 1962, Haacke 1964, 1975). This feature is most strongly developed in the sand-living *P. kochi* whereas *P. carpi* has the least developed fringes, presumably because extensive fringing is not necessary to dig in course substrates. The more extensive fringes in *P. kochi* also probably aid in walking on soft sand (Brain 1962, Haacke 1964, 1975). Additional adaptations of *Ptenopus* for a subterranean lifestyle include extra-brillar fringes to keep soil out of the eyes (these can cover the eyes completely, like mammalian eyelids) and projections to cover the nostrils from the inside, which are absent or reduced in *P. carpi* (Bellairs 1948, Brain 1962, Haacke 1964, 1975). *Ptenopus* species have been referred to as "burrowing geckos" similar to multiple other species in southern Africa (Haacke 1975), but are more permanently subterranean than other species.

Burrows are key in nearly all aspects of the lives of *Ptenopus*: they are refuges during the day and much of the night, the males call from the burrow entrances, courtship interactions occur at the burrow entrance, mating and egg-laying takes place within the burrows, and prey is often taken at or near the burrow entrance. A burrow is usually occupied by an individual for months at a time and the occupant rarely travels far from the burrow entrance (Haacke 1969, 1975, Gigard 1997, Polakow 1997, Hibbitts et al. 2005, 2007, Hibbitts 2006). *Ptenopus kochi* shows a tendency for males, females, and juveniles of a few weeks old to share burrows, which tend to have more than one entrance (Haacke 1969). By contrast, *P. garrulus* tend to be solitary burrow occupants (Hibbitts et al. 2005). Both *P. garrulus* and *P. kochi* appear to lay single eggs per clutch, while gravid *P. carpi* females generally contain two well-developed eggs (Haacke 1975, Hibbitts et al. 2005). Less is known about the ecology of *P. carpi* than the other two species.

In all species, males call from the burrow entrance to attract females and establish territories (Haacke 1969, Polakow 1997, Hibbitts et al. 2007, 2012). These exceptionally loud calls are produced using well-developed vocal chords in combination with tracheal modifications, and the unusually deep head may be used as a resonating chamber (Rittenhouse et al. 1998, Russell et al. 2000). Territorial behaviour has only been observed in *P. kochi* and *P. garrulus*, whereby a calling male will either retreat from, or attack another calling male within about a metre of the burrow entrance (Polakow 1997, Hibbitts et al. 2007, 2012). Similarly, courtship is better-known in these two species. In *P. garrulus,* the female approaches the calling male with few or no notable courtship interactions above-ground. Mating takes place within the burrow, and the male leaves in about 30 minutes to dig a new burrow nearby (Hibbitts 2006). In *P. kochi*, courtship may involve some mutual

head bobbing and a gular patch display by the male, and the female sometimes inspects the male burrow prior to accepting the male as a mate (Polakow 1997). The pair then live together in the burrow for extended periods of time and the young also remain in the burrow for several weeks after the female has vacated (Polakow 1997). Male vocalisations are therefore used to advertise not only the availability of their genes to females, but also a home and an oviposition site.

There are clear differences in the calls among the three species of *Ptenopus* (Brain 1962, Haacke 1969, Gramentz 2008). In light of species-specific differences in the advertisement calls of other geckos (Rohtla et al. 2019) and a wide range of well-studied vocal taxa (Blair 1974, Loftus-Hills and Littlejohn 1992, Robinson and Hall 2002, Hobel and Gerhardt 2003, Bailey et al. 2017, Jaya et al. 2022), this trait is undoubtedly important for mate recognition. This is likely to be especially important where *Ptenopus* species occur sympatrically.

Haacke (1969) recorded substantial variation in the calls of *P. garrulus* from different geographic locations. Observations on a few captive *P. garrulus* individuals from two geographically distant (but unspecified) locations suggested that females respond to the calls of a male from the home population, but not to those from the geographically distant location (Gigard 2002). This supports the notion of mate recognition and potentially unrecognised species divergence within *Ptenopus garrulus*. Haacke (1975) also identified differences in the coarseness of lepidosis in geographically disparate populations of *P. garrulus,* particularly among *P. g. maculatus* populations occurring on different soils. This adds evidence to suggest potentially unrecognised species contained within *P. garrulus*. However, Haacke (1969; 1975) did not favour the interpretation of call variation as indicating population or species divergence. This was partially due to his failure to match calls with individual specimens and partially because he was unable to separate the variation in calls caused by environmental variation, and that potentially caused by genetics. Calls uttered late at night or at colder temperatures were slower, lower-pitched, and had fewer notes or pulses (Haacke 1969). Haacke even suggested that a single individual may utter more than one call, or that calls may be behaviourally learnt from parent populations, rather than being genetically fixed.

No thorough revision of *P. garrulus* call variation has been attempted since the last taxonomic revision of the calls (Haacke 1969), despite considerable advances in our understanding of bioacoustic analyses, the application of theory, and call recording equipment since the 1960s. Moreover, the formal call description of *P. carpi* (Gramentz 2008) still rests on a total of two calls, recorded from one individual in captivity. There is clearly a need to revise the calls of *Ptenopus* with modern sound analysis techniques, taking into account the theoretical concepts and implications of mate recognition on advertisement calls, in a phylogenetic and systematic context.

There has also been no comprehensive phylogenetic analysis done on *Ptenopus*, and no systematic revision since Haacke (1975). Its phylogenetic placement within Gekkonidae has been investigated (e.g., Feng et al. 2007, Gamble et al. 2012), and while it is not closely related to other gecko genera in southern Africa, its closest known relative on the subcontinent is *Narudasia festiva* (Pyron et al. 2013). Sequences from only two individuals are available on GenBank, and the relationships of the three species have not yet been assessed. Moreover, there has been no attempt to investigate the potential for cryptic or unrecognised species in the genus. This is particularly important to do for the most widespread species, *P. garrulus,* considering the observed intraspecific variations in morphology, call, and habitat. In this thesis, I conducted the first thorough investigation of phylogenetic variation for the genus *Ptenopus*, and thoroughly revised the variation in advertisement calls in a systematic context. I investigated various potential drivers of divergence and reassess the taxonomy, ecology, distribution, and habitat of the genus in light of these findings.

CHAPTER 2: Substrate specialisation drives ecological diversification in the African barking geckos (*Ptenopus*: Gekkonidae)

Abstract

Barking geckos (genus *Ptenopus*) are a group of terrestrial, burrowing lizards endemic to southern Africa, with three recognised species. Two species are range-restricted (*P. kochi* and *P. carpi*) and display clear differences in substrate preference (soft sand vs. hard gravel). The third and most widespread species, *P. garrulus,* occurs on a range of substrates, across a range of potential geographic barriers, and along a steep and wide climatic gradient. Variations in morphology and advertisement call in this species indicates that it may be a species complex. I produce the first comprehensive phylogeny for the genus and test the hypothesis that *P. garrulus* is a species complex. I also tested various candidate hypotheses of divergence drivers in the genus, namely ecological specialisation onto different substrates, vicariance scenarios, and climatic niche specialisation. The phylogeny supported multiple deeply divergent lineages within *P. garrulus*, while *P. kochi* and *P. carpi* were monophyletic but also contained genetic substructure. The distribution of the major clades coincided with different levels of substrate hardness and with bioclimatic ecoregions. Generalised linear models supported substrate specialisation over other factors as the key driver of divergence in the genus. Geographic barriers, including rivers and mountains or ridges, were supported as drivers of some of the more recent divergences. Overall, the results support a primarily adaptive radiation in *Ptenopus* with a smaller non-adaptive component, as opposed to the primarily non-adaptive radiations recorded for most rupicolous geckos in the same region.

Introduction

Southern Africa is a recognised hotspot for the evolution of lizards, with notable radiations in the Gekkonidae and Lacertidae in the arid western portion of the subcontinent (Bauer 1993, 1999, Herrmann and Branch 2013, Heinicke et al. 2017, Garcia-Porta et al. 2019, Conradie et al. 2022). These arid landscapes are characterised by a patchwork of rock formations, sand dunes, and various arid plains (Atlas of Namibia Team 2022a, 2022b). Specialisation onto the various substrates has played a pivotal role in these lizard radiations. Rupicolous species inhabit fragmented rock islands separated by a matrix of plains habitat, which has resulted in mainly non-adaptive radiations

(Heinicke et al. 2017, Conradie et al. 2022). Substrate generalists and species inhabiting the continuous and widespread terrestrial habitats, usually exhibit lower rates of diversification (Heinicke et al. 2017).

Ptenopus (barking geckos) is a relatively widespread genus, inhabiting various plains habitats (as opposed to rocky or mountainous habitats) across xeric Southern Africa. Members of the genus are uniquely vocal, the males producing loud advertisement calls in large choruses around sunset (Smith 1849, Haacke 1969). Only three species of *Ptenopus* are recognised: *P. kochi* and *P. carpi* are endemic to the Namib Desert in western Namibia, while *P. garrulus* is much more widespread. There are two subspecies of *P. garrulus* recognised, with *P. g. maculatus* inhabiting the most arid south-western portion of the range, whereas *P. g. garrulus* occurs in the semi-arid savannahs further east (Haacke 1975).

Substrate hardness is an important habitat component for *Ptenopus* because these geckos create elaborate burrow systems within which much of their lives are spent, and mating and egg-laying take place (Haacke 1975, Polakow 1997). I use the term 'hardness' to refer to the properties of dry soils, which is related to soil 'texture', although the latter refers to the properties of damp soils. *Ptenopus* have specialised hand and foot morphologies for digging in dry soils. Strong nails on the fingers are for excavation, while rows of fringed scales along the lateral margins of all toes aid in scooping loose soil backwards (Brain 1962, Haacke 1975, Polakow 1997). Toe fringes have evolved multiple times among lizards including geckos, and are usually associated with burrowing or moving within windblown sand (Luke 1986, Bauer and Russell 1991). As in other lizard groups, the extent of these fringes are linked to substrate hardness (Haacke 1964, 1975) with the two extremes being evident in the two substrate specialists: *P. kochi* has the most extensive fringing and is restricted to the soft sand of the Namib Sand Sea, whereas *P. carpi* occurs only on hard gravels and has the least extensive fringing of the three species. The widespread *P. garrulus* has intermediate toe fringing (Haacke 1975). Whether this can be interpreted as a generalist adaptation for versatile substrates, an adaptation for soils of intermediate hardness, or whether other factors influence the extent of toe fringes is unclear.

In the west, *P. g. maculatus* appears to be a substrate generalist, its range overlapping with both other species and extending across a range of substrate types. By contrast, *P. g. garrulus* in the eastern portion of the distribution appears to specialise on the Kalahari Arenosols, occurring on interdune spaces, vegetated dunes, and on the more widespread sandy flats. In the last revision of the genus, Haacke (1975) found variation in the coarseness of lepidosis (or number of body and head scales) in *P. g. maculatus*, which he interpreted as evidence of different populations or lineages linked to different substrates. By contrast, *P. g. garrulus* occurs on the widespread Kalahari

Arenosols and displays little morphological variation in geographically distant populations. Moreover, there is variation in the advertisement calls of *P. garrulus* (Haacke 1969), which is often linked to different species in various vocal groups, including among geckos (Rohtla et al. 2019). Additionally, the other two *Ptenopus* species have distinct calls (Haacke 1969, Gramentz 2008). It is therefore likely that *P. garrulus* and particularly *P. g. maculatus,* is a complex of species specialising on different substrates, rather than a widespread substrate generalist.

In Namibia, I identified three substrate transition zones where soil hardness changes drastically over a short geographic distance, with soft sand giving way to hard gravels or calcic soils in less than a hundred metres. These transition zones provide opportunities to robustly test the hypothesis of *P. garrulus* as a species complex of substrate (soil) specialists, with divergent genetic (metapopulation) lineages being expected to occupy the two adjacent substrates at each transition zone. By contrast, geographically distant samples from the same substrate (soil of the same hardness), would be expected to be from the same genetic lineage, isolated by distance. The first two transition zones are at the northern and southern borders of the Namib Sand Sea, western Namibia (see Fig. 1): A – the Gobabeb Transition Zone, across the Kuiseb River; C – the Lüderitz Transition Zone, between the coastal and northern soft sands and southern or eastern hard soils. A third transition zone was identified in south-eastern Namibia, at the south-western edge of the Kalahari Sand Sea near Koës: B – the Koës Transition Zone (see Fig. 1). There are several other places across southern Namibia and the northern Cape where different soil types also meet, although the difference in hardness is not as drastic as at the three transition zones identified.

If different substrates (specifically soils of different hardness) present different habitats for *Ptenopus*, then wide stretches of uninhabitable substrate would present barriers to dispersal. *Ptenopus* do not inhabit mountainous or rocky substrates (multiple personal observations and analysis of available georeferenced records), hence wide stretches of these substrates are also likely to present barriers to dispersal. Within the range of *Ptenopus* these include the Khomas Highlands in central Namibia, the Etendeka volcanic formation in north-western Namibia, the Nama Group formation in south-western Namibia, the greater Otavi Mountain Range in northern Namibia, and the Great Escarpment, from western Namibia through western and southern South Africa. A number of these have also been suggested to be barriers for other lizard species (e.g., Bauer et al., 2006; Childers et al., 2021; Lamb and Bauer, 2000; Makokha et al., 2007; Scott et al., 2004).

Other potential barriers to dispersal within the range of *Ptenopus*, particularly those suggested by studies on lizard taxa, include the Orange River and the Kuiseb River (Lamb and Bauer 2000, Scott et al. 2004, Bauer et al. 2006, Makokha et al. 2007, Childers et al. 2021). The former may be a substantial barrier due to its likely Cretaceous age (Dingle and Hendry 1984). The latter is

untestable as a barrier because it marks the northern border of the Namib Sand Sea. The sand sea is expected to be a more substantial barrier to *Ptenopus* and to other taxa, than the (ephemeral) river itself. Several westward-flowing rivers along the Namibian west coast also transect the distribution of *Ptenopus garrulus* and *Ptenopus carpi* without a marked difference in substrate on the northern or southern banks and can therefore be tested as potential barriers. These rivers are ephemeral, but may have flowed more substantially and permanently during the Pleistocene when deep river incisions were made (Korn and Martin 1957). Hippopotamus tusks found in the Swakop River (Irish 2012) may also suggest a past perennial flow, although these have not been dated. These rivers may therefore have resulted in past cladogenesis, but are unlikely to present current boundaries to dispersal.

Finally, several climatic factors may be expected to drive or facilitate divergence in *Ptenopus*. I focus on two climatic categorisations which affect ecoregion- or ecosystem-level changes in fauna and flora (Olson et al. 2001, Sayre et al. 2013, Dinerstein et al. 2017). The first is the divide between primarily summer and winter rainfall zones (Neukom et al. 2014), with only the south-westernmost *Ptenopus garrulus* populations occurring in the latter zone. The second is a steep gradient or progressive change in bioclimate, thermotype and ombrotype eastwards from the subcontinental west coast (see Sayre et al., 2013). This gradient is caused by the Atlantic Benguela cold water current which causes regular foggy, cold, windy, and generally hyper-arid conditions in the coastal Namib Desert, and a steep climatic gradient eastward from this zone (Atlas of Namibia Team 2022c).

In this manuscript, I produce the first full phylogeny for the genus *Ptenopus*, including samples from across the ranges of all three species to reassess its diversity, identify previously overlooked radiations, and test the hypothesis that *P. garrulus* contains cryptic species. The sampling design was carefully constructed to include samples from different soil types, across geographic barriers and substrate transition zones, and from different bioclimatic zones. Thus, I was able to robustly test various hypotheses of divergence drivers against each other.

Under the hypothesis that *P. garrulus* consists of cryptic lineages specialised on different substrates, it was expected that genetically divergent clades would occur across the substrate transition zones (identified *a priori*), but that geographically distant samples on a similar substrate would represent a single lineage. Mantel tests were used to distinguish between clades that represented distinct lineages and those which merely constituted part of a single lineage isolated by distance (IBD). A novel genetic-geographic mapping approach based on Mantel regressions, was then used to identify potential barriers or areas across which elevated levels of divergence occurs. Finally, I tested the various candidate drivers of divergence against each other using multiple regression on matrix models (MRMs). These candidate drivers included geographic distance which

was the ‘null-model’ (i.e., not separate lineages, but a single lineage under IBD), substrate or soil hardness (occurrence on), climatic gradient, rainfall seasonality, and vicariance due to various geographic barriers identified.

Methods

Sampling, study area, and soil classification

Barking geckos were located using a T16 LED Lenser torch to spot eye-shine at night, and/or by their vocalisations at dusk. Tissue samples were taken as tail tips and preserved in 99% ethanol, NAP buffer (EDTA, Sodium citrate, Ammonium sulphate buffer) or in solution of 20% dimethylsulphoxide saturated with NaCl.

The geckos were sampled from across the range of each *Ptenopus* species in Namibia and South Africa, so as to cover a variety of soil types. “Soil texture” refers to the properties of moist soils (USDA 2016), but *Ptenopus* dig their burrows in dry soils. Therefore, soil surface “hardness” while dry, is a more appropriate classification of *Ptenopus* habitat. No formal classification exists for assessing this, so careful notes in the field were compared with soil type data according to the Harmonised World Soil Database (Fischer et al. 2008) and the Atlas of Namibia (Atlas of Namibia Team 2022b). Soil type, which is related to soil texture class, appeared to be a relatively good indicator of soil hardness, although this can vary somewhat on a single soil type. Soil hardness was therefore very simply classified into three categories based on how easily the soil surface could be disturbed or excavated: ‘soft’, ‘intermediate’, and ‘hard’. ‘Soft’ soils are always Arenosols, wherein a person will leave clear, deep (>5 mm) footprints. ‘Intermediate’ soils will not leave deep footprints, but can be easily excavated with the bare hand, whereas ‘hard’ soils are difficult or impossible to excavate by hand, having usually a hard, cemented surface crust and/or course, gravelly texture. On a landscape scale, I used data from the Harmonized World Soil Database (Fischer et al. 2008) and the Atlas of Namibia (Atlas of Namibia Team 2022b), with certain soils classified under one of the three categories (Table 1).

Table 1: Soil classification for sampled locations

Classification	Soil types included	Observational criteria	General description
'soft'	Arenosols	A person leaves clear, deep (>5 mm) tracks	Wind-blown or finer-textured river sand, with no or very little cementation or compaction of any kind
'intermediate'	Regosols; Cambisols; compacted Arenosols	No deep tracks, but easy to excavate with bare hand	Soils containing mostly sand, but some level of cementation or compaction is present, often courser-grained sands or mixed soils, and often having some vegetation cover
'hard'	Calcisols; Durisols; Gypsisols; compacted Regosols; deeper/softer pockets within Leptosols	Not easy or impossible to excavate with bare hand	Gravelly soils and/or soils with a hard crust, i.e. considerable surface cementation or compaction present

Three major substrate transition zones in terms of soil hardness were identified prior to sampling, to test whether different clades would occur on the adjacent soils of different hardness (see Fig. 1):

A) Gobabeb Transition Zone: the area near Gobabeb Research Station (approx. -23.5, 15.4) in the central Namib Desert, soft Arenosols of the Namib Sand Sea occur on the south-western side of the Kuiseb River, while hard gravel plains (Gypsisols) occur on the north-eastern side. On the southern side of the Kuiseb, inter-dune plains are mostly inhabited by *P. g. maculatus* while the dunes themselves are usually inhabited by *P. kochi*.

B) Koës, transition zone: the area around Koës, Namibia (approx. -25.8, 19.3) with the soft Kalahari Sand Sea (Arenosols) to the northeast and the hard Calcisols to the southwest.

C) Lüderitz transition zone: the area between Lüderitz and Aus, Namibia, with soft Arenosols in and at the edges of the Namib Sand Sea (the tall dunes are uninhabited by *Ptenopus*), and along the coast, and intermediate (compacted Arenosols and Regosols) or hard (including Durisols and compacted Regosols) plains further east (inland), south of the Sand Sea.

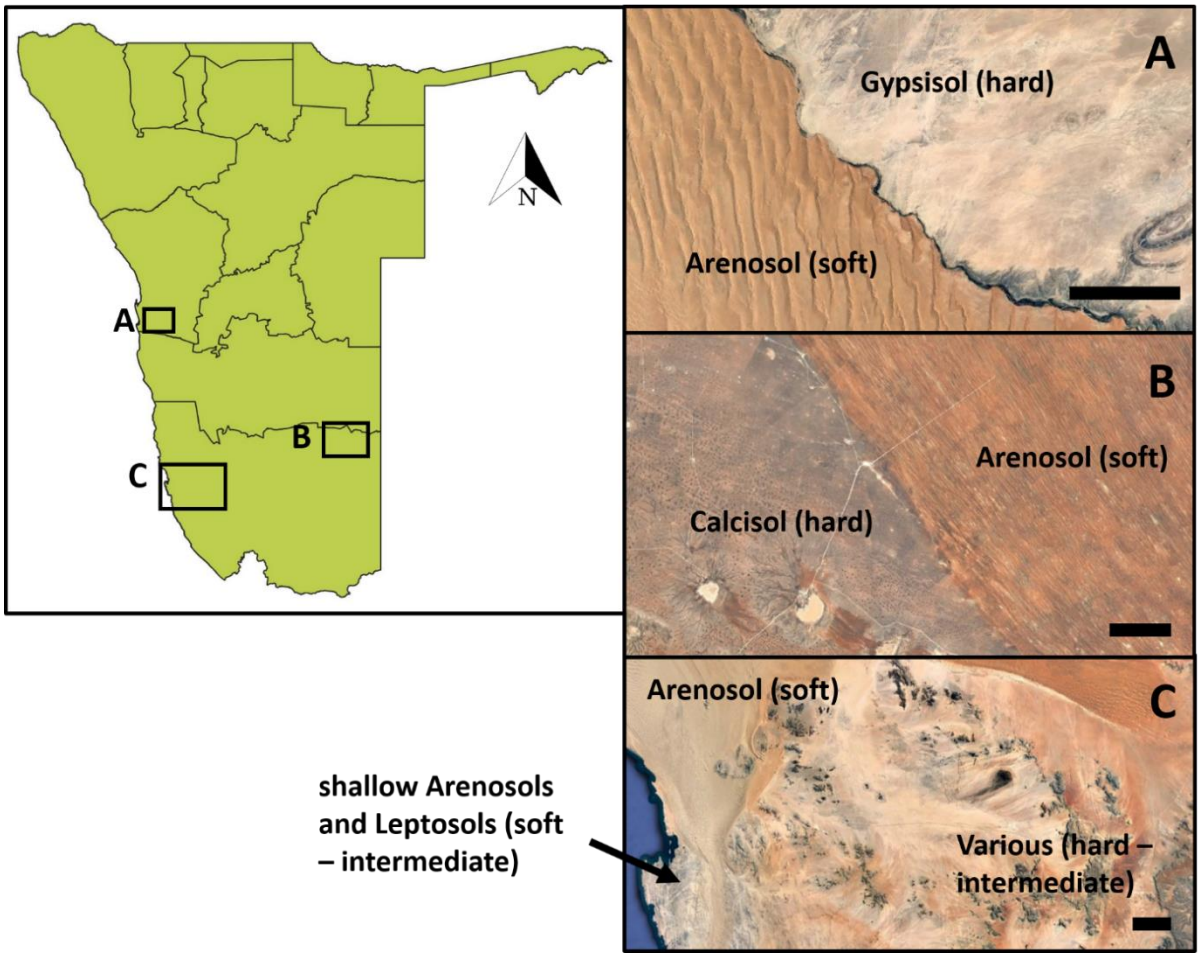

Figure 1: The three substrate transition zones (A – C) in Namibia, identified prior to sampling. A = Gobabeb Transition Zone, B = Koës Transition Zone, and C = Lüderitz Transition Zone. Different soil types and their hardness category are indicated as text, and black scale bars represent 10 km.

DNA extraction, amplification and sequencing

A total of 79 *Ptenopus* samples from a broad geographic range and across all three recognised species were analysed (Appendix 1: Table S 1). Two *Narudasia festiva* samples were included as the closest outgroup taxon – see (Pyron et al. 2013).

Tissue samples were rinsed three times with deionised water and dried in a vacuum centrifuge prior to DNA extraction. Total genomic DNA was extracted using a salt extraction protocol (Aljanabi and Martinez 1997) or with the Qiagen DNeasy Blood & Tissue Kit following the manufacturer's instructions. PCR amplification of two mitochondrial markers (*ND2* and *16S*) and one nuclear marker, oocyte maturation factor MOS (*c-mos*) were carried out using the following forward and reverse primer sets: for *c-mos*, FU-F and FU-R (Gamble et al. 2008); for *16S*: 16Sa and 16Sb (Palumbi 1996); for *ND2*: vMet2 and vTrp (Cunningham and Cherry 2004). PCR cycling conditions were as follows: *16S*: denaturation (95 °C, 4 min), 30 cycles of denaturation (94 °C, 45 s), annealing

(51 °C, 45 s), extension (72 °C, 1 min.), and final extension (72 °C, 10 min). For *ND2*: denaturation (95°C, 4 min.), 38 cycles of denaturation (95 °C, 35 s), annealing (55 °C, 45 s), extension (72 °C, 1 min), and final extension (72 °C, 10 min). For *c-mos*: denaturation (95 °C, 4 min.), 30 cycles of denaturation (94°C, 45 s), annealing (50 – 60 °C varied by sample, 45 s), extension (72 °C, 40 s), and final extension (72 °C, 10 min). For some samples, annealing temperatures and denaturation times were adjusted to achieve amplification. PCR amplicons were visualised by electrophoresis on a 0.8% agarose gel with ethidium bromide. Sanger sequencing was carried out at Macrogen (Amsterdam, The Netherlands) using the forward primers for each gene.

Phylogenetic analyses

Sequences were checked for quality, edited, and aligned in Geneious 2022.1.1 (Kearse et al. 2012). Alignments were inspected manually noting protein translation, to confirm the absence of stop codons for coding regions and to remove misaligned gaps in the hypervariable region of the mitochondrial 16S ribosomal RNA gene.

The program jModelTest 2.1.10 (Guindon and Gascuel 2003, Darriba et al. 2012) was used to assess the evolutionary model that best fitted each of the partitions using the Akaike information criterion (AIC) test and confirming similar results with the Bayesian Inference Criterion (BIC) test. This was incorporated into the Bayesian phylogenetic analysis (parameters for *16S* and *ND2*: GTR + I + G model, nst = 6, rates = gamma, ncat = 4; for *c-mos*: HKY + I model, nst = 2, rates = equal). Bayesian inference (BI) and maximum likelihood (ML) analyses were performed on the combined dataset. The Partition Homogeneity Test in PAUP (Swofford 2001) was used to test for topological congruence between the nuclear and mitochondrial trees. Mr. Bayes 3.2.7 (Huelsenbeck and Ronquist 2001) was used for the Bayesian analysis, through the Cyberinfrastructure for Phylogenetic Research (CIPRES) Science Gateway v3.3 (www.phylo.org/portal). The ML analysis was carried out with the command line local version of RAxML-VI-HPC v7.0.4 (Stamatakis 2006). Divergences among clades were estimated in MEGA-X (Kumar et al. 2016) using uncorrected net p-distances for each of the three genes on all available ingroup individuals.

A total of 79 individuals were included in the ingroup (Appendix 1: Table S 1), with two *Narudasia festiva* samples as the most closely related outgroup (see Pyron et al. 2013). All individuals were represented with an *ND2* sequence and at least one additional sequence of *c-mos* or *16S.* The total evidence dataset consisted of 2,016 base pairs (bp), with 14.5% missing data including the missing sequences for some individuals: 16S = 550 bp (6.6% missing data plus 4 missing sequences); *ND2* = 1050 bp (9.3% missing data); *c-mos* = 416 bp (5.5% missing data plus 24 missing sequences).

Mantel tests and spatial regression

Mantel tests were conducted on the mitochondrial dataset (more available samples and more appropriate mutation rates than nuclear *c-mos* gene) to test for isolation-by-distance (IBD) within or among the major clades. A second iteration of Mantel tests was also conducted on subclades with >3 geographically disparate samples. Both raw and log-transformed geographic distances were used for each model fit, and the fit with a better R^2 value was used.

Residuals from these fitted regressions, plotted as genetic-geographic connectivity maps (GGCMs), were used to identify potential geographic barriers, directionality of greater or lesser gene-flow, and lineage splits in geographic space. The GGCMs are geographic maps of the sample locations, indicating sample pairs with notably greater- or lower-than-expected p-distance given the geographic distance, based on residuals from the fitted Mantel regression. Sample pairs that deviate substantially from the fitted regression were flagged, with positive deviation indicating greater-than-expected divergence or genetic isolation between samples, and *vice versa* for negative residuals. The residual standard error (RSE) was generally used as a threshold for sample pairs to be flagged as outliers, although some datasets showed wide dispersion of the data, in which case values deviating less than the RSE were also inspected.

Multiple regression on distance matrices (MRMs)

A robust approach for testing various candidate hypotheses for drivers of genetic divergence against each other was developed to maximise the strengths of the robust geographic and taxonomic sampling design applied in this study, using multiple regression on distance matrix (MRM) models.

The partial Mantel test is commonly used to investigate the relationship between genetic p-distance and an explanatory variable, while keeping the variation explained by geographic distance constant (Smouse et al. 1986). However, a multiple linear regression approach is more versatile and has better power for detecting linear relationships (Lichstein 2007, Legendre and Fortin 2010). Moreover, with sufficient data points, not just one or two, but multiple relationships with sequence divergence can be tested against each other, simultaneously.

MRMs were therefore used to test for a positive relationship between sequence divergence (p-distance, the response variable) and various explanatory variables, which were all converted into distance matrices. Thus, it was possible to test the validity of different candidate hypotheses for drivers of genetic divergence against each other, and against the null-model of IBD (positive relationship with geographic distance). These candidate hypotheses were: difference in soil hardness (between sample pairs), difference in climatic gradient, difference in summer/winter rainfall zone,

and vicariance caused by different potential barriers. These models were conducted on the whole genus, on groups of clades (e.g., *P. garrulus*) and on each of the major clades.

Distance table matrices (among relevant samples) for all explanatory variables were generated in R (4.1.0, R Core Team 2021) using packages ape (Paradis and Schliep 2019), seqinr (Charif and Lobry 2007), raster (Hijmans 2022), and using base-R. To avoid model over-fitting, only one variable per candidate hypothesis was used for model-selection. Full models including all candidate variables/hypotheses were fitted in using function 'glm' in R. The function 'step' was then used to select the AIC-best model in terms of complexity. The R package 'relaimpo' (Grömping 2006) was used to calculate the total percentage explained by the AIC-best model and by each explanatory variable, as an average of two alternative but similar R^2 metrics developed for GLMs (Genizi 1993, Zuber and Strimmer 2011). The AIC model selection was the first step to test among candidate hypotheses, and relative variable contributions the second step. If a candidate hypothesis is supported, this would be reflected by a strong positive relationship with p-distance.

The full models included geographic distance (km, between sample pairs) as the explanatory variable for IBD – effectively the null-model. The explanatory variable soil hardness was scaled as 1 = soft, 2 = intermediate, and 3 = hard, and the difference in these values between all samples pairs was calculated, with 0 = same soil hardness, 1 = slightly different hardness, 2 = most different hardness. Difference in distance from the west coast (km) was used as a proxy for (difference in) position along the climatic gradient. Difference in primarily summer/winter rainfall zone was scaled as a binomial factor, with 0 = same zone, 1 = different zones. The final variable was vicariance, scaled as a binomial factor for which samples across the hypothesised barrier were scaled as 1 = separated, vs. 0 = in contact (on the same side of the barrier). If more than one potential barrier was relevant for a particular clade or group of clades, alternative barrier models were included in alternative full models (e.g., including all barriers, only one barrier, or a combination of some barriers). The AIC-best barrier model (if any) was then retained. Once the AIC-best model was chosen, the R^2 for each variable was estimated to quantify relative variable importance, as variation in p-distance explained.

Results

Molecular phylogeny

Both the maximum-likelihood and the Bayesian phylogeny recovered seven major well-supported clades within *Ptenopus*, defined by the deepest nodes in the topology (Fig. 2), henceforth "major clades", or clades named as in Fig. 2. The species *P. kochi* and *P. carpi* and the subspecies *P. g. garrulus* were recovered as monophyletic clades, but subspecies *P. g. garrulus* was paraphyletic with multiple clades that are not necessarily sister clades. The major clade containing *P. g. garrulus* also contained one minor clade previously assigned to *P. g. maculatus* (the southernmost clade). Three of the major *P. g. maculatus* clades (*P. g. maculatus* Central, South, East) were affiliated to *P. g. garrulus* and the fourth (*P. g. maculatus* North) was sister to *P. kochi* with good support. *Ptenopus carpi* was recovered basally divergent in the Bayesian analysis (Fig. 2), whereas the maximum likelihood analysis recovered *P. carpi*, *P. kochi* and *P. g. maculatus* North clades as a poorly supported (likelihood = 47%) sister clade to *P. g. garrulus* and affiliated *P. g. maculatus* clades. Various subclades were also identified and referred to where relevant. The nuclear and mitochondrial topologies were congruent, with no evidence for significant difference between the topologies (p = 1.000).

There was a notable gap between intra- and inter-clade p-distances for all major clades, with a few exceptions. Among the major clades, mitochondrial p-distance (Table 2; Table 3) was lowest between clades *P. g. maculatus* South and East (8% for *ND2* and 3.5% for *16S*), compared to higher intra-clade p-distance (8.8% for *ND2* and 4.3% for *16S*) within clade *P. g. maculatus* South. The lowest interclade p-distance in nuclear *c-mos* (Table 4) was between clades *P. g. garrulus* and *P. g. maculatus* South, and also between *P. g. maculatus* Central and East, compared to the higher (and highest) intra-clade divergence within clades *P. g. garrulus* and *P. g. maculatus* Central (0.14% and 0.18%, respectively).

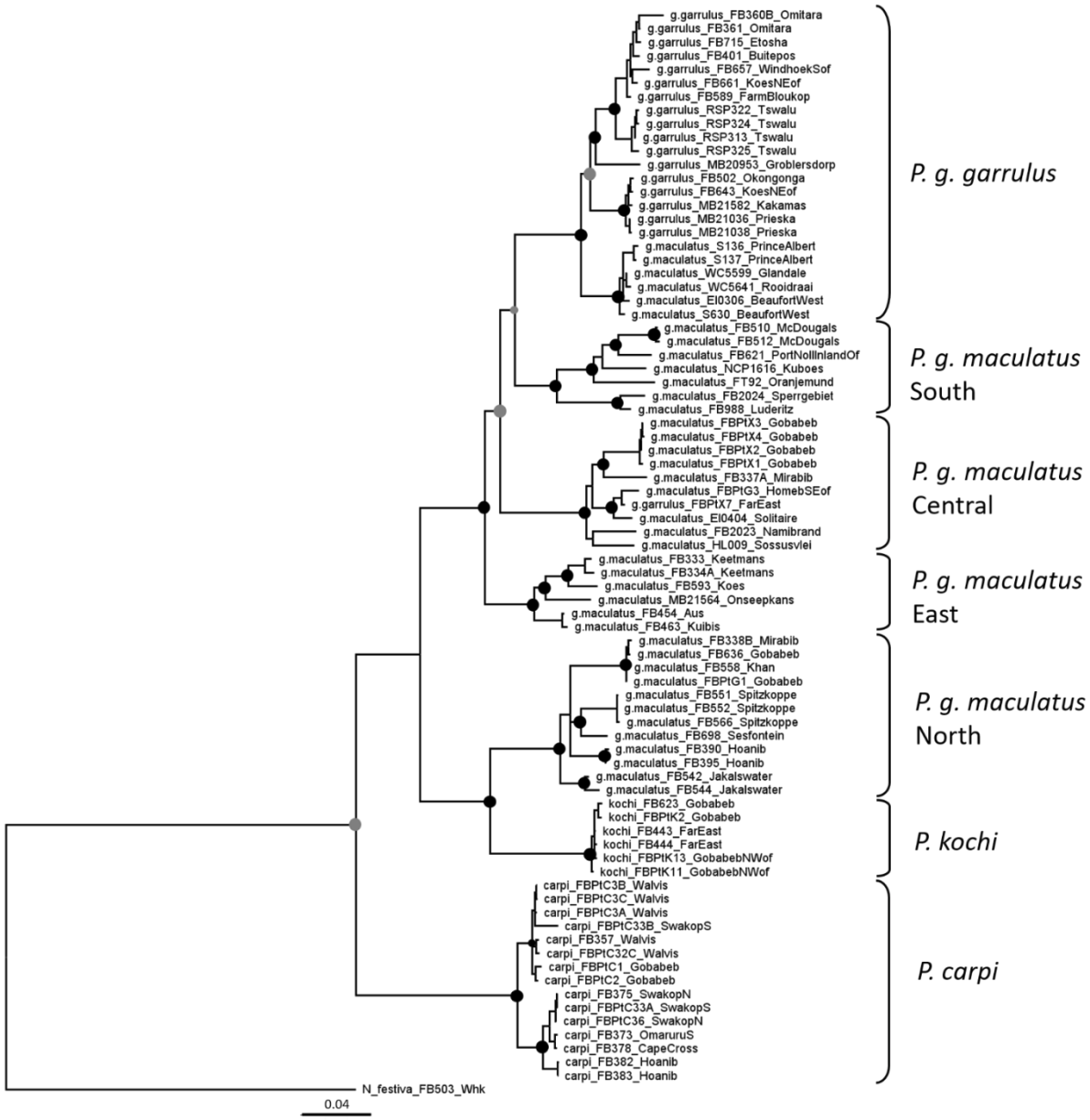

Figure 2: Bayesian topology of *Ptenopus* based on a concatenated dataset of *16S*, *ND2*, and *c-mos* genes, with *Narudasia festiva* as the outgroup. Nodes supported by both maximum likelihood (≥70% bootstrap) and Bayesian (≥0.95 posterior probability) analyses are denoted with black circles and by Bayesian only, with grey circles. Nodes supported ≥65% bootstrap and/or ≥0.9 posterior probability are similarly shown but using smaller circles. Node support values close to branch tips are not shown. Labels to the right indicate the major clades identified as some of the deepest supported nodes in the phylogeny.

Table 2: Inter- and intra-clade uncorrected pairwise net sequence divergence (%) for the seven major clades of *Ptenopus,* for ND2 mitochondrial gene. Intra-clade p-distance higher than lowest interclade distance (8%) shown in boldface. Clade names are presented in a shortened format using only specific epithet, or area for *P. g. maculatus* clades (as *m.* Area).

	Intra-clade	*carpi*	*garrulus*	*m.* Central	*m.* East	*m.* South	*m.* North
carpi	3.9						
garrulus	6.1	18.8					
*m. C*entral	6.0	19.1	10.3				
m. East	6.4	18.4	9.0	9.1			
m. South	**8.8**	17.7	8.2	8.5	8.0		
m. North	6.0	16.7	15.7	14.9	14.2	13.9	
kochi	0.5	21.3	20.2	19.5	18.8	17.6	13.7

Table 3: Inter- and intra-clade uncorrected pairwise net sequence divergence (%) for the seven major clades of *Ptenopus,* for 16S mitochondrial gene. Intra-clade p-distance higher than or equal to the lowest interclade distance (3.5%) indicated in boldface. Clade names are presented in a shortened format using only specific epithet, or area for *P. g. maculatus* clades.

	Intra-clade	*carpi*	*garrulus*	*m.* Central	*m.* East	*m.* South	*m.* North
carpi	0.5						
garrulus	2.4	10.0					
*m. C*entral	2.8	9.7	6.3				
m. East	2.9	9.3	5.1	5.9			
m. South	**4.3**	8.2	5.0	4.8	3.5		
m. North	**3.5**	10.0	9.6	8.8	7.4	7.2	
kochi	0.6	13.2	11.6	10.4	9.1	9.0	4.3

Table 4: Inter- and intra-clade uncorrected pairwise net sequence divergence (%) for the seven major clades of *Ptenopus,* for nuclear *c-mos* gene. Unusually low and high (overlapping) inter- and intra-clade p-distances, respectively, shown in boldface; for one incalculable inter-clade net p-distance, mean inter- p-distance is displayed with an asterisk. Clade names are presented in a shortened format using only specific epithet, or area for *P. g. maculatus* clades.

	Intra-clade	*P. carpi*	*garrulus*	*m.* Central	*m.* East	*m.* South	*m.* North
carpi	0.00						
garrulus	**0.14**	0.80					
*m. C*entral	**0.18**	0.70	0.25				
m. East	0.00	0.99	0.25	***0.09**			
m. South	0.00	0.76	**0.05**	0.27	0.26		
m. North	0.00	1.00	0.32	0.55	0.52	0.27	
kochi	0.00	1.27	0.57	0.62	0.77	0.53	0.26

Distribution and substrate characteristics of the major clades

There was an overall distributional partitioning into different soil hardness categories among the major clades. At the substrate transition zones, two (Fig. 3: B & C) to four (Fig. 3: A) major clades occurred parapatrically on the two different substrates. At the Gobabeb Transition Zone (A), *P. g. maculatus* North and *P. carpi* occurred sympatrically and syntopically on the hard gravel plains north of the river, whereas *P. kochi* and *P. g. maculatus* Central occurred sympatrically and syntopically on soft sands south of the river. At the Koës Transition Zone (B), *P. g. garrulus* occurred on the soft Kalahari Sand Sea, whereas *P. g. maculatus* East occurred on the hard Calcisols adjacent. At the Lüderitz Transition Zone (C), *P. g. maculatus* South occurred on soft to intermediate coastal sands, whereas *P. g. maculatus* East occurred on intermediate to hard compacted soils (compact Regosols and Durisols) further inland.

In the Namib Desert clades, some partitioning with distance from the coast was also evident. For instance, *P. carpi* was rarely recorded more than 40 km inland, whereas *P. g. maculatus* North rarely occurred closer than 40 km to the coast. Similarly, *P. g. maculatus* South rarely occurred more than 40 km from the coast and *P. g. maculatus* East rarely closer.

Across all recorded localities within all major clades (including many observations not tabled), occurrence on a single substrate was nearly ubiquitous, with some notable exceptions. However, the exceptional cases mostly represented genetically divergent populations (see Appendix 2).

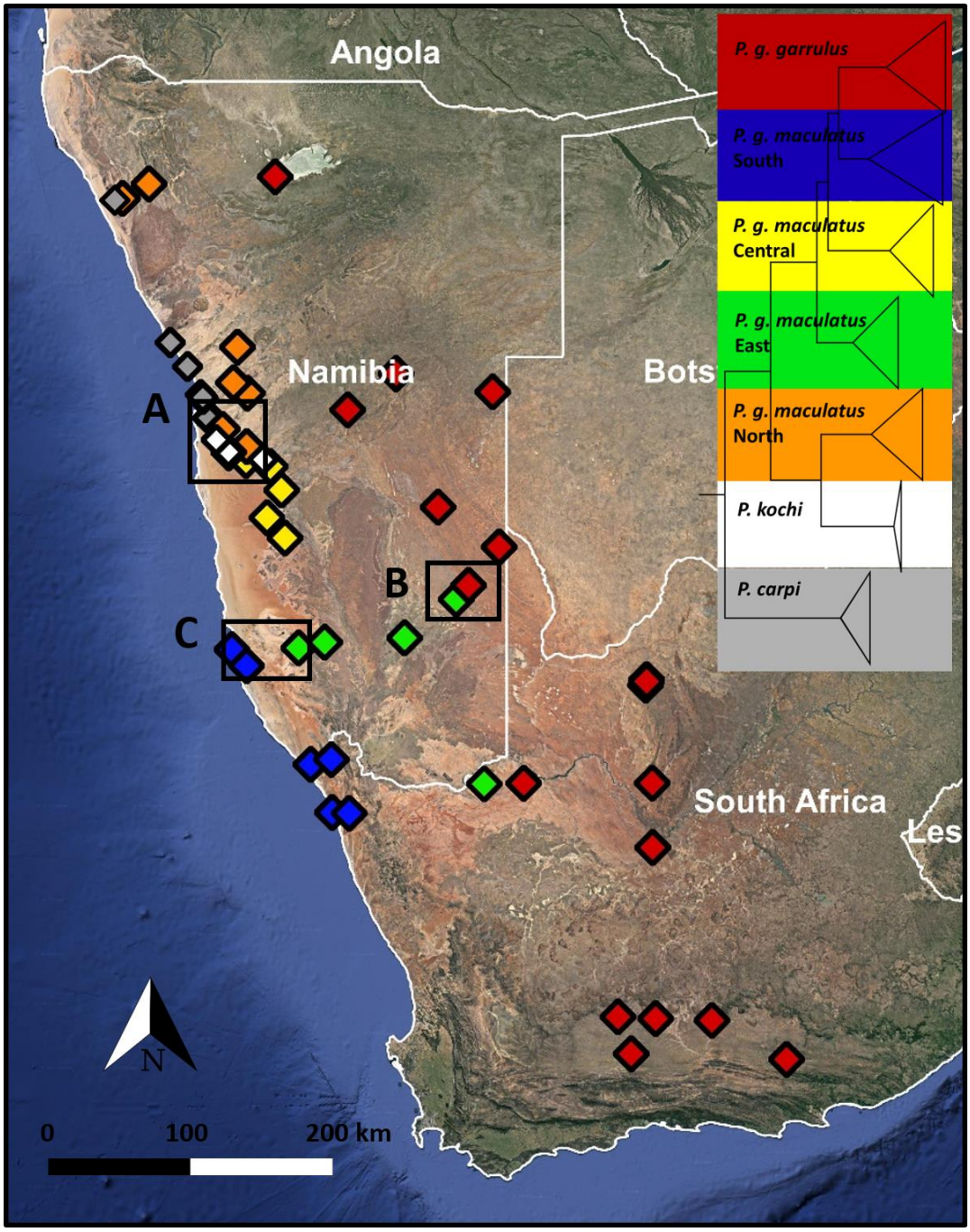

Figure 3: Map of *Ptenopus* sample locations, with the approximate areas of the three substrate transition zones (A = Gobabeb Transition Zone, B = Koës Transition Zone, C = Lüderitz Transition Zone) indicated by labelled black frames. A simplified extract of Fig. 2 shows the phylogeny with coloured clades (labelled on extract) matching the colours of the records on the map.

Mantel tests and genetic-geographic connectivity maps

Mantel tests and GGCMs support the major clades as separate genetic lineages rather than a single grouped lineage isolated by distance. *Ptenopus garrulus*, if analysed as a single species, showed a very poor fit to the IBD model. If analysed as separate clades, these showed much higher R^2, except for clade *P. g. garrulus* which only showed moderately higher R^2 (Table 5). Clades *P. g. maculatus* South, Central, and East, which showed low interclade divergence in either *16S* or *ND2* mitochondrial genes (Tables 2 – 4), when analysed together showed moderately high and significant R^2 (Table 5). However, if analysed separately all three clades showed a considerably higher R^2 (Table 5), and geographically closest samples among these clades were genetically highly divergent (Appendix 2: Fig. S 1). Clades *P. g. garrulus*, *P. kochi,* and *P. carpi* displayed significant but low R^2, and *P. g. maculatus* North an intermediate and significant R^2, indicating a poor to moderate fit to the IBD model in these clades.

The genetic-geographic connectivity maps (see Appendix 2) detected elevated levels of divergence within the major clades, across some of the hypothesised barriers including tracts of unsuitable soils, some mountain ranges, the Orange River, the Great Escarpment, and the Kuiseb River. Distance from the coast appeared to be important in some coastal clades. Some additional potential barriers were identified, including Cenozoic rocky deposits or ridges in the Northern Cape, South Africa, and the Swakop River in the central Namib Desert, Namibia. Some of the hypothesised barriers including the Khomas Highlands and the Otavi Highlands, appeared not to be barriers (see Appendix 2: Fig. S 2). All the potential barrier scenarios were included in the GLM model-fitting.

Table 5: Mantel test for isolation by distance within each of the seven major clades of *Ptenopus*, with some clades lumped. 'Model' indicates whether raw or log-transformed geographic distance was used. Values for R^2 and p-values are given, with asterisks indicating significant or highly significant p.

Clade or group	**Model**	**R^2**	**P**	
P. garrulus: all clades	y~log(x)	0.19	<0.001	**
P. g. garrulus	y~x	0.21	<0.001	**
P. g. maculatus South + Central + East	y~log(x)	0.57	<0.001	
P. g. maculatus South	y~x	0.85	<0.001	**
P. g. maculatus Central	y~log(x)	0.76	<0.001	**
P. g. maculatus East	y~x	0.70	0.006	**
P. g. maculatus North	y~log(x)	0.37	<0.001	**
P. kochi	y~log(x)	0.20	0.055	*
P. carpi	y~log(x)	0.22	<0.001	**

Multiple regression on (distance) matrices (MRMs)

The multiple regression on (distance) matrix models (MRMs) indicate that substrate-based barriers and substrate category explain most of the variation in p-distance within *P. garrulus* as a whole, followed by geographic distance, while rainy season only explained 1% variation in p-distance (Table 6).

Among the major *Ptenopus* clades there were various different combinations of most important explanatory variables (Table 6). These included substrate hardness, geographic distance, and various barriers including the Orange, Kuiseb, and Swakop Rivers, the Great Escarpment, and some ridges in the Northern Cape (Table 6). Distance from the west coast was of minor importance among some of the coastal or near-coastal clades (Table 6).

Table 6: AIC-best MRM results, based on distance tables of p-distance vs. explanatory variables, of various *Ptenopus* clades. 'Group/clade' refers to the major clades in Fig. 2 and 'Var. explained' is the total percentage variation in p-distance or divergence explained by the model. 'Variables' are the explanatory variables in the current model, which include a combination of geographic distance (km), one or more barriers to dispersal (binomial variable), distance from the west coast (km), and/or substrate hardness category (1 – 3). For each explanatory variable the p-value ('P') and R^2 (proportion of p-distance variation explained) is indicated.

Group/clade	**Var. explained**	**Variables**	**P**	**R^2**
P. garrulus: all clades	36%	log(geo distance)	< 0.001	0.09
		substrate	< 0.001	0.11
		barrier: substrate	< 0.001	0.15
		rainy season	< 0.001	0.01
P. g. garrulus	47%	log (geo distance)	<0.001	0.13
		barrier: scarp & ridge	<0.001	0.34
P. g. maculatus South	91%	geo distance	< 0.001	0.70
		substrate	0.048	0.04
		distance W coast	0.042	0.01
		barrier: Orange R	0.166	0.16
P. g. maculatus Central	81%	log (geo distance)	< 0.001	0.73
		barrier: Kuiseb R	0.001	0.08
P. g. maculatus East	88%	geo distance	< 0.001	0.60
		substrate	0.001	0.28
P. g. maculatus North	51%	log(geo distance)	0.003	0.23
		distance W coast	0.130	0.05
		barrier: Swakop R	0.000	0.23
P. kochi	69%	substrate	< 0.001	0.69
P. carpi	53%	log(geo distance)	0.153	0.07
		distance W coast	0.052	0.04
		barrier: Swakop R	< 0.001	0.42

Discussion

The phylogenetic analyses uncovered a previously undetected radiation of the genus *Ptenopus*. The phylogeny supported seven deeply divergent clades (henceforth "major clades" or as specific clade names), with *P. kochi* and *P. carpi* being monophyletic. As expected, cryptic diversity was most evident in the widespread *P. garrulus*, although contrary to expectations, this species was paraphyletic, with one major clade being sister to *P. kochi. Ptenopus* is far more diverse than current taxonomy suggests, with divergence among the major clades being comparable to species-level divergences in other reptiles including geckos (e.g., Agarwal et al., 2014; Branch et al., 2021; Busschau et al., 2019; Castiglia and Annesi, 2011), and substantial intraclade divergences within these major clades.

There was strong evidence for substrate specialisation among the major clades. At the three substrate transition zones identified *a priori*, it was expected that samples from the two adjacent substrates of different hardness would be from genetically divergent clades, whereas geographically distant samples on the same substrate would be from the same clade or lineage. This was found to be the case at all three transition zones, with two major clades occurring on the two substrates in parapatry. This was also evident at a fourth transition zone identified at the southern edge of the Kalahari sand sea. Occurrence on the same or similar substrates within a single major clade was nearly ubiquitous. The distribution of the major clades appeared to be mostly determined by substrate, specifically soil hardness.

It was well known that *P. kochi* and *P. carpi* are specialists on soft and hard soils respectively, but the widespread *P. garrulus* was previously thought to be a substrate generalist. The phylogeny recovered five major clades within *P. garrulus,* four of which are within the subspecies *P. g. maculatus,* occurring on different substrates, with one highly divergent clade being sister to *P. kochi.* The major clade *P. g. garrulus* also contains one subclade currently assigned to *P. g. maculatus*, being the southernmost population of *Ptenopus* (Rebelo et al. 2018). The subspecies *P. g. garrulus* is genetically less diverse and geographically far more widespread than the *P. g. maculatus* clades, occurring on Arenosols in and around the greater Kalahari. These findings are consistent with suggestions of Haacke (1975) who observed morphological variation in *P. g. maculatus* populations on different substrates, whereas *P. g. garrulus* displayed less morphological variation across the range. However, Haacke (1975) only suggested one potential species-level split (linked to substrate) – in the central Namib, between the clades *P. g. maculatus* North and *P. g. maculatus* Central. Furthermore, his morphological "populations" did not fully coincide with the various clades identified here.

Haacke (1975) did not record marked differences in the toe fringing among his morpho-populations, and simply classified the toe fringes of *P. garrulus* overall as “intermediate”. However, I observed more extensive toe fringes and wider (hand/foot) palms/soles for the clades living on softer substrates, particularly in *P. g. garrulus* and *P. g. maculatus* Central. There also appeared to be variation of this trait within the major clades, with populations on notably looser sand displaying more extensive fringing than those on slightly more level or compact sand, although quantifying this trait was not within the scope of this study. Toe fringing in *P. garrulus* clades was never as extensive as in *P. kochi,* but the substrate inhabited was also not as soft. My categorisations of soil hardness were mainly focussed on substrates inhabited by *P. garrulus*, while the sand inhabited by *P. kochi* is even softer than the “soft” category discussed here. The typical habitat for *P. kochi* is the loose, sloping sand on a dune plinth, whereas the sympatric *P. g. maculatus* Central, for instance, occurs on the flat sand of the interdunes. Clear differences in the advertisement calls were also noted for the different major clades of *P. g. maculatus*, but quantifying these was beyond the scope of this study. The geographic distribution of the various clades indicate that substrate specialisation played a key role in the radiation of *Ptenopus.*

The MRMs support the hypothesis that substrate specialisation was the main driver of divergence among the *P. g. garrulus* group of clades. This was tested against the alternative hypotheses of geographic distance or IBD, climatic gradient, summer/winter rainy season, and various potential geographic barriers including the Great Escarpment, the Orange River, and even several major mountain ranges (these models were not well-supported). Geographic distance also had moderate support from the models and rainy season was of minor importance (Table 6).

The importance of substrate-based vicariance as a driver of divergence was probably overestimated by the MRMs, because they can only assess the current spatial pattern of divergence. This may be problematic when piecing together the deepest divergences, because clades may have moved far from the original position of cladogenesis in the proceeding time. Substantial substrate barriers only occurred between highly divergent clades, and not closest sister-clades, which usually occur on adjacent soil-bodies of different hardness. This indicates that the colonisation of a novel substrate type probably drives the initial divergence event. Subsequently, habitat tracking or movement of members of a lineage across the subcontinent, may lead to spatial separation of geographically distant clades over evolutionary time. The likely substantial movement of individuals belonging to a particular clade after initial divergence, is clear among the more closely related *P. garrulus* clades. Clade *P. g. maculatus* East is sister to all the geographically surrounding major clades, with sister-clades currently distributed on either sides of it (Fig. 3). Drivers of divergence

among major clades is probably best assessed through environmental correlates to distribution, while shallower divergences can be more accurately assessed with this MRM approach.

Climatic drivers, such as distance from the west coast and summer/winter rainy season were not important explanatory variables for divergence among the major clades of *P. garrulus* as a whole and could not be assessed for the whole genus in a MRM. However, considering that clade *P. g. maculatus* South occurs entirely within the winter rainfall zone and several clades or species occur only within a narrow range of distances to the coast, these factors probably have played an important role in driving, or at least facilitating, divergence in *Ptenopus*.

Substrate specialisation was generally not important among the shallower divergences within the major clades, because in most clades very little to no variation in substrate was recorded. Where such variations occurred, these usually coincided with elevated genetic divergence (given the geographic distance). In clade *P. kochi*, soil hardness was the only explanatory variable in the AIC-best model (not even geographic distance was included in this model), explaining 69% of variation in p-distance among samples, resulting from one divergent population found on harder soils on the northern banks of the Kuiseb River. In *P. g. maculatus* East, soil hardness was moderately important (28% variation) while geographic distance explained most of the variation (60%). Soil hardness explained only 4% of the p-distance variation in clade *P. g. maculatus* South, and was not included as an explanatory variable in any of the other clades, where very little or no soil hardness variation was recorded. This indicates that the event of colonising a novel substrate is relatively rare in evolutionary time, and clades are highly conserved in their substrate preference.

Contrary to the pattern of divergence among deeper clades, geographic barriers including rivers, ridges and the Great Escarpment, were likely key drivers of the shallower divergences within most of the major clades. The Orange River as a barrier explained 16% of divergence within *P. g. maculatus* South, whereas most of the remaining p-distance variation (70%) was due to geographic distance, and distance from the west coast was of minor importance (1% p-distance variation). In clade *P. g. garrulus,* the southern escarpment separates the southern subclade within the major clade *P. g. garrulus,* from the rest of the major clade in the north (Appendix 2: Fig. S 2), and a few rocky ridges of Cenozoic deposits separate the divergent Groblersdorp sample from the geographically closest surrounding samples. Together, these barriers explained 34% of the total divergence in this clade. Geographic distance (log-transformed) only explained a further 13%, while the substantial remaining divergence (47%) could not be explained by any of the included variables. Two subclades within the major clade *P. g. garrulus* occur in broad contact and occasional sympatry throughout the Kalahari Sand Sea (see Appendix 2: Fig. S 2), which may reflect a divergence event caused by past conditions or barriers that no longer persist. Within clade *P. g. maculatus* Central, the

Kuiseb River (see Appendix 2: Fig. S 4) as a barrier was supported over the alternative hypothesis of substrate-driven divergence, although these two hypotheses are difficult to separate because the Kuiseb River is also the northern border of the sand sea and causes a major change in substrate (soil hardness). Even though the Kuiseb River is dry nearly throughout the year, the upper reaches of the Kuiseb Canyon consist of wide tracts of exposed rock, which are uninhabited by *Ptenopus* and certainly add credibility to this finding.

The Swakop River as a barrier was supported as the most important driver of divergence in both clades which occur across this river: clade *P. carpi* and *P. g. maculatus* North, the latter displaying three minor clades occurring across the Swakop and Khan Rivers (the Khan River is a tributary of the Swakop River: see Appendix 2: Fig. S 6; Fig. S 8). This ephemeral river probably does not present a current barrier to *Ptenopus,* as evidenced by occurrence of both these clades within the riverbed, and by one sample from the southernmost subclade of *P. g. maculatus* North having been found north of the Swakop River and one individual from the northern subclade of *P. carpi* having been found to the south. It is possible that the Swakop River flowed more perennially in the past and have presented a barrier then (see Korn and Martin 1957).

Perhaps surprisingly, several potential barriers to dispersal were not supported by the MRMs. For instance, the Namibian portion of the Great Escarpment and the wide Etendeka Formation in Namibia did not explain divergence patterns in the clades traversing these vast rocky habitats. Even though these rocky features are largely unoccupied by *Ptenopus,* a network of small patches or channels of suitable substrate could provide sufficient corridors for genetic cohesion of *Ptenopus* populations that occur in this area. The Khomas and Otavi Highlands are broad, substantial mountain ranges traversing the range of clade *P. g. garrulus*, and are certainly devoid of *Ptenopus*. However, the vast expanse of available substrates east of these mountains appears to have facilitated efficient gene flow of *Ptenopus* around these barriers, since these were not supported as barriers by the MRMs. This seems not to have been the case around the much smaller rocky ridges near Groblersdorp, South Africa (see Appendix 2: Fig. S 2), which are supported as a barrier by the MRMs.

The radiation of *Ptenopus* is clearly linked to substrate specialisation. While this pattern is similar to other southern African lizards, most species in this region are rupicolous, displaying primarily non-adaptive radiations driven by vicariance (e.g., Mouton and Van Wyk 1990, Jacobsen 1994, Bauer 1999, Heinicke et al. 2017, Conradie et al. 2022). By contrast, the more continuous nature of terrestrial substrates resulted in a primarily adaptive radiation of *Ptenopus*, with divergence events probably caused by the colonisation of a novel substrate in contact with a previously occupied substrate. The distribution patterns of the different clades on different

substrates is exceptionally 'neat' compared to most terrestrial lizards. This pattern bears some similarity to that of *Meroles* species, which are also distributed on distinctive patches of substrate of different 'hardness' (see Branch, 1998; Lamb and Bauer, 2003). *Ptenopus* appears to be highly morphologically conserved, which likely led to the underestimation of species diversity in the past. However, my observations suggest that differences in advertisement call may show promise for species identification among the morphologically more similar lineages identified here.

Conclusions

My results support a previously undocumented radiation within the genus *Ptenopus,* with multiple lineage divergences at various stages of progress. The deepest divergences are characterised by a primarily adaptive radiation, with specialisation on soils of different hardness and the colonisation of novel soil types being the major cause of cladogenesis. Soil hardness specialisation appears to be conserved within the major clades, with only a few deviations recorded. These deviations normally represented subclade divergences. Younger radiations within the major clades were primarily non-adaptive, with several rivers and narrow mountain ridges causing vicariance. The central Namib Desert in Namibia is the centre of diversity for the genus, containing four of the most deeply divergent major lineages, and also an active site of current evolution. Multiple divergent clades, some deeply divergent and others very recent, occur adjacent to one another on the two sides of the Kuiseb River, with soft sand on one side and hard gravel on the other. The genetic, ecological and spatial analyses presented here, clearly support the hypothesis that *Ptenopus* contains unrecognised species. However, the extent of lineage divergence to be considered species-level needs to be explicitly assessed. This will require a case-by-case consideration of the various lines of evidence provided here, in conjunction with analyses of morphological variation and variation in advertisement calls.

The GGCM mapping approach and the application of MRMs to evaluate biogeographic hypotheses used here, are powerful methods for exploring spatial patterns of divergence and for robustly testing biogeographic hypotheses against each other. Moreover, the MRM approach can be applied for testing phenotypic relationships with genetic variation, similar to Robles-Bello et al. (2022). While similar methods have been applied to landscape genetic community analyses, most commonly in microbes (e.g., Ning et al. 2020, Zuo et al. 2022), this approach has not previously been applied in the biogeography context presented here.

CHAPTER 3: Speciation, mate recognition, and reproductive character displacement in barking geckos (*Ptenopus*: Gekkonidae)

Abstract

The barking geckos (*Ptenopus*) are an unusually vocal gecko genus endemic to southern Africa. Although only three species are recognised, a recent phylogenetic analyses revealed a remarkably diverse radiation in this genus. I applied the unified species concept to delimit species within *Ptenopus,* using multiple lines of evidence including genetic-spatial analyses, advertisement calls, and ecology, to provide evidence for evolutionarily distinctiveness among various clades hypothesised to be species. I also investigated reproductive character displacement in the advertisement calls using multiple regression on matrix models (MRMs) to relate call divergence to genetic divergence, while accounting for sympatry. My results strongly supported eight species within the genus, with moderate support for further species-level splits. My results also showed strong evidence for reproductive character displacement in the calls, with genetic divergence explaining 38%, and allopatry vs. sympatry explaining 10% of the divergence in advertisement call traits. This is the first study to show evidence for reproductive character displacement of vocal traits in a non-avian reptile.

Introduction

Among the squamates, geckos are the only group which possess true vocal chords and the ability to produce sounds with tonal qualities (Gans and Maderson 1973, Werner et al. 1978, Moore et al. 1991). Many species emit advertisement calls, usually produced by the male (Rohtla et al. 2019). Barking geckos, genus *Ptenopus*, are arguably the world's most vocal geckos (Haacke 1969). Males produce impressively loud advertisement calls, chorusing together in their thousands in some situations. As Smith (1849) remarked in the species description of *P. garrulus*: "and the number thus occupied is at times so great, and noise so disagreeable, as to cause the traveller to change his quarters". Their calls are particularly notable in the Namib Desert, where *Ptenopus* is particularly common and its calls occupy much of the biological soundscape.

Advertisement calls, like other courtship signals, tend to be under stabilising selection within a population, but become divergent among genetically divergent populations or species

(Lande 1981, Endler and Houde 1995, Butlin and Ritchie 2009). This is mainly due to coevolution, which usually results from the necessary compatibility between the male signal and the female reception of that signal, within a population or species (Butlin and Ritchie 2009). The appreciation of vocal signals in geckos has received little attention compared to other vocal taxa such as birds, bats, and anurans, partially because most gecko calls are very soft. *Ptenopus* is one of the notable exceptions, and some of the earliest works on species specificity of gecko advertisement calls were conducted on *Ptenopus* (Brain 1962, Haacke 1964, 1969, Gramentz 2008). It is now more widely appreciated that gecko advertisement calls, like most vocal taxa, are species-specific (Rohtla et al. 2019).

If different species occur sympatrically, there is strong selection for being able to discriminate between the courtship signals of hetero- and conspecifics, which leads to the evolution of mate recognition systems (Paterson 1985, Boake et al. 1997, Köhler et al. 2017). According to the reinforcement hypothesis (Dobzhansky 1937, Howard 1993), species may initially diverge allopatrically which can result in some divergence of mate recognition traits. If the two divergent species come back into geographic contact, selection against hybridisation causes these traits to become more different so as to maintain reproductive isolation between two closely related species in allopatry. The observed pattern is generally referred to as reproductive character displacement, although this pattern can arise for reasons other than reinforcement, for instance due to competition for signal space in advertisement calls (Hoskin and Higgie 2010). Very few studies have shown strong evidence for reproductive character displacement, or indeed for the reinforcement hypothesis, with notable case studies on advertisement calls in anurans (Blair 1974, Loftus-Hills and Littlejohn 1992, Hobel and Gerhardt 2003, Jaya et al. 2022), plumage in birds (Sætre et al. 1997), and dewlaps in *Anolis* lizards (Webster and Burns 1973, Lambert et al. 2013). Evidence for reproductive character displacement in vocalisations has not been demonstrated in geckos or any non-avian reptile. Due to their unusually vocal nature and the clear link to courtship, the barking geckos are an ideal group to study this phenomenon.

There are currently three described species of *Ptenopus*: *P. carpi* Brain, 1962, *P. kochi* Haacke, 1964, and *P. garrulus* (Smith 1849). *Ptenopus garrulus* has two subspecies, *P. g. garrulus* and *P. g. maculatus* Gray, 1866. My phylogenetic analysis of the genus (Chapter 2) found that species diversity within *Ptenopus* is underestimated by the current taxonomy. Seven clades, henceforth "Chapter 2 clades", were identified and shown to be ecologically divergent, specialising on soils of different hardness (Chapter 2). The species *P. kochi* and *P. carpi* and the subspecies *P. g. garrulus* each represented separate clades, whereas the subspecies *P. g. maculatus* is made up of four paraphyletic clades. I also identified notable genetic structure within all of these seven clades,

caused by geographic distance, various geographic boundaries, climatic gradients, and (smaller) differences in substrate specialisation. In this chapter, I re-evaluate the taxonomic model of *Ptenopus,* considering various lines of evidence to delineate species within *Ptenopus*.

The advertisement calls in *Ptenopus* provide an ideal model to apply the concepts of mate recognition and reinforcement to the species delimitation process. Audible differences in advertisement call had been noted in the different clades and different locations, but these differences have not yet been quantified. It is expected that different species would exhibit divergent bioacoustic characters of the advertisement calls, but that the same traits would be relatively conserved within a species due to stabilising selection (e.g., Bailey et al. 2017, Blankers et al. 2017, Minter and Du Preez 2017). Moreover, if advertisement calls play a key role in maintaining reproductive isolation among species of *Ptenopus*, then displacement of these traits due to reinforcement are expected among sympatric species.

In this chapter, I considered four operational criteria or lines of evidence to delimit species in the genus *Ptenopus* under the unified or general lineage species concept (GLC: de Queiroz 1998, 2007): phylogenetic, ecological, morphological (as either phenetic cluster or diagnostic, fixed difference) and recognition (mainly based on advertisement calls). First, I used species delimitation analyses to suggest putative species limits using the phylogeny. I then evaluated the validity of putative species based on these lines of evidence. Finally, I considered candidate species supported by at least three of these four lines of evidence (including, necessarily, phylogenetic-spatial evidence).

As part of the delimitation process, I developed a bioacoustic framework to compare *Ptenopus* call characters. I hypothesised that there will be reproductive character displacement of advertisement calls among the sympatric candidate species, and stabilising selection of call traits within a species. I tested these hypotheses using multiple regression on matric models (MRMs). Under these hypotheses, I expected calls to be more different between individuals from sympatric species given the amount of genetic divergence (per sample pair), than between allopatric species, and least different among conspecifics, even given sequence divergence.

Methods

Application of species concept

The unified or general lineage species concept (GLC: de Queiroz 1998, 2007) was used to evaluate genetic clades as species within the genus *Ptenopus*. Under this concept, four operational criteria or lines of evidence were used to assess whether a putative species is supported as a separately evolving metapopulation lineage: phylogenetic, ecological, (mate) recognition, and morphological (either as quantitative phenetic cluster, or diagnosable fixed difference). I took a conservative stance in delimiting species using this integrative taxonomic framework (Padial et al. 2010), considering only species supported cumulatively by at least three operational criteria.

Field sampling: calls and morphology

Geckos were located using a T16 LED Lenser torch to spot eye-shine at night, and/or by their vocalisations at dusk. Male advertisement calls of each *Ptenopus* species were recorded with digital recorders including Olympus WS–802, Bell DVR–6006, and N28 Professional HD Intelligent, at a resolution of 16 bit and sampling rate of 48 kHz, at a distance of ~0.6 m from the calling males. The recorders were placed at the burrow entrance of a calling male with a small crescent of soil around as a wind shelter. DS1922L/T/E/S Temperature Logger iButtons were placed behind the burrow entrance as a proxy for operative temperature of a gecko calling with its head out of the burrow. Geckos were subsequently captured from the burrows or while foraging, photographed using a Nikon D3100 camera with 18-55 zoom lens and 22 mm Meike digital zoom extension tubes. Photographs included various angles (usually dorsal, ventral, and lateral) of the entire gecko, the head, the hands and feet, the vent, and around the body, marking the gecko on the white belly with a marker pen (to keep count of scales), with a ruler for scale, for morphometric analyses. The snout-vent length (SVL) and total length (TL) were also measured before release.

Phylogenetic species delimitation

The major clades from Chapter 2, henceforth “Chapter 2 clades”, were used as a preliminary clade structure for putative species and compared to the results from the two species delimitation analyses. A single-locus mitochondrial dataset (*16S* and *ND2* genes) was used to conduct species delimitation analyses for *Ptenopus* as most of the analyses were designed for single-locus data. Congruence with the total-evidence phylogeny (including nuclear *c-mos*) was inspected in each case. The validity of candidate species from these delimitation approaches were further tested in light of other lines of evidence (sections below).

First, the automatic barcode gap detection (ABGD Puillandre et al. 2012) was performed via the ABGD web interface (web version '11/18/2022'). Simple p-distance metric was selected, with priors for minimum relative barcode gap width = 1 and (default) intraspecific divergence minima (0.001) and maxima (0.1).

Second, a Bayesian general mixed Yule-coalescent model (bGMYC) was implemented using the package bGMYC v. 1.0 (Carstens and Reid 2012) in R (v 3.2.4, R Core Team 2016). A dataset of trees to sample for this analysis was estimated by Bayesian Inference in BEAST 2.6.6 (Bouckaert et al. 2019) on the mitochondrial dataset, through the Cyberinfrastructure for Phylogenetic Research (CIPRES) Science Gateway v3.3 (www.phylo.org/portal). The HKY site model with model parameters estimated for each gene using jModel test (Darriba et al. 2012), and the relaxed log normal clock model with default primers were implemented at 100,000,000 iterations, sampling every 5,000. The bGMYC analysis was applied to 1,000 posterior trees, randomly sampled from 50,000,000 initial trees after 50% burn-in from the concatenated BEAST runs, implementing 1,000,000 MCMC steps with 100,000 steps as burn-in and sampling every 100 steps. Because bGMYC tends to over-split species (e.g., Miralles and Vences 2013), the more conservative estimates from this analysis (lower probability thresholds in bGMYC output) were used.

Third, the phylogenetic evidence was interpreted in a spatial context (see Cicero et al. 2021, Tolley et al. 2022) to provide the final support for putative species from the phylogenetic operational criterion. Results from multiple regression on matrix models (MRMs), Chapter 2, were re-interpreted in light of the identified putative species in this chapter; some additional MRMs were fitted using the same methods as Chapter 2, on novel configurations of putative species as suggested by the species delimitation analyses. The approach was used as follows: two putative sister species as single dataset, were tested for isolation-by-distance (IBD), i.e., that genetic divergence (p-distance, the response variable) among the samples from both putative sister species, could be sufficiently explained by geographic distance. In this cases the split was considered "not supported". In relevant cases, alternative explanatory variables (to geographic distance) including likely geographic barriers, soil hardness, climatic gradient, or climatic zone were fitted in addition to geographic distance (see Chapter 2 Methods). If geographic distance was a poor explanatory variable, and/or poorer than an alternative explanatory variable of genetic divergence, the split was considered as "supported" by phylogenetic evidence. Sequence divergence between putative species was estimated in MEGA-X (Kumar et al. 2016) using uncorrected net mean p-distances for all three genes evaluated in Chapter 2 (mitochondrial *ND2* and *16S;* nuclear *c-mos*).

Relevant evidence from the MRMs (from Chapter 2) was also used to support ecological speciation. If soil hardness or climatic drivers explained a substantial amount of sequence

divergence between putative species, this was interpreted as evidence of evolutionary independence under the ecological operational criterion.

Bioacoustic analyses

Significant differences in advertisement calls were interpreted as the primary line of evidence for mate recognition (GLC operational criterion), as a system for two putative species to maintain reproductive and hence evolutionary independence. See methods sections on PCAs and MRMs, below, for an interpretation of these data.

A total of 839 calls from 98 individuals, sampled from all seven Chapter 2 clades and from multiple different localities or populations, were analysed (Appendix 3: Table S 2). Only calls from gene-sequenced populations were included in this study. No more than 10 calls per individual were included, usually the first ten. Call variables were averaged per individual and then averaged again per species or clade.

I based the call metrics measured and terminology used on the recommendations developed by Köhler et al. (2017) for frog taxonomy. Some of these metrics are comparable to those previously used for *Ptenopus* (Haacke 1969, Gramentz 2008), but even these metrics were slightly differently applied. Previous literature described the calls as a series of “clicks”, “squeaks” (Haacke 1964, 1969), or “syllables” (Gramentz 2008). I use the standardised term “notes” (as defined in Köhler et al. 2017), harmonious with broadly applied bioacoustic terminology, to describe this component.

Haacke (1969) noticed that calls sound different (lower and slower, sometimes fewer notes) late at night and/or at lower temperatures. I confirmed this observation by recording several individuals throughout the night, while measuring temperature. Calls from outside the main chorus period (late at night and/or in cold temperatures) were therefore excluded. However, I was able to determine (through many calls recorded from the same individuals) that the note duration and inter-note interval increase or decrease proportionally, if the call becomes faster or slower due to varying temperatures. I therefore developed several relative timing call metrics (described below) to provide more consistent measurements of the calls, which are not substantially influenced by varying temperatures.

The call variables measured (including new metrics, defined) were: number of notes (n notes); note repetition rate (s^{-1}); median note duration (ms); note 1 duration deviance (difference between the first note duration and the median note duration, in percentage of the median); median inter-note interval (ms); inter-note interval range (as a percentage of the median inter-note interval: a measure of variability); median call density (median note duration divided by median inter-note interval, no units, with “denser” calls having longer notes and shorter intervals); call

duration (s); upper and lower (where relevant) dominant frequency (kHz) – most *Ptenopus* species have two frequency peaks of similar amplitude, hence both are termed 'dominant', although the upper peak (or amplitude peak of higher frequency) of the two is usually louder, especially at closer recording distances; basal frequency (Hz); bandwidth, or upper and lower 90% (of peak amplitude) bandwidth frequencies (kHz); call period (call duration plus following inter-call interval).

Individual calls were cut as separate WAV files and analysed with a customised code (Appendix 3: Code S 1) using the program R (R Core Team 2016), including packages soundgen (Anikin 2019) and seewave (Sueur et al. 2008). Program Audacity (Audacity Team 2019) was used to analyse aspects of some calls which could not be properly estimated using the R-script (usually because of other call interference, noise, or the call being too soft).

Morphology

Under the GLC, a phenetic cluster (quantitative) or diagnosable, fixed difference (qualitative) can be used as operational criteria. I used either approach as supporting evidence for evolutionary independence under the morphological or phenotypic operational criterion.

Photographs of newly collected material (from gene-sequenced populations) and physical specimens of various putative species housed in the collections of the National Museum of Namibia (NMNW), Windhoek, Namibia, were examined to identify useful diagnosable morphological characters for some of the putative species identified in this chapter (Appendix 4: Table S 3 and additional specimens). A number of morphological characters (including number of fringes on fingers and toes, number of labial scales, proportions of rostral and mental scales, scales bordering mental, number of scales between the eye and nostril, number and shape of internasal scales between the prenasal scales) and measurements were inspected for the *P. garrulus*-group species, but did not show promise for taxonomic diagnosis.

While *P. kochi* and *P. carpi* are already easily diagnosable using morphology, the putative species included in *P. garrulus* are morphologically highly conserved. After a thorough exploration of various features, only a few diagnosable characters were retained and assessed for these putative species: the number of scales in contact in a straight line across the top of the head between the eyes, in line with the mid-pupil of the pupils – interorbital scales (IOS) and the number of midbody scale rows (MBSR). The following relative measurements (in pixels) were taken using ImageJ (Schneider et al. 2012), on a single full frontal photograph of the gecko's snout (see Appenix 3: Fig. S 9): 1) nostril (horizontal diameter) breadth (NB); 2) internarial distance, from the nearest edges of the nares (IN); 3) rostral (horizontal) breadth at the lower edge of the lip (RB); 4) rostral (vertical) height (RH); 5) horizontal breadth of the base of the largest inter-nasal scale in contact with the

rostral (INSBB); 6) vertical height of the largest inter-nasal scale (INSH). Supplemented data of IOS and MBSR counts were extracted from Haacke (1975), including only data from the localities close to gene-sequenced populations. The raw per-specimen data were not available, so data were generated from a normal distribution using the sample size, mean, standard deviation, and range supplied by Haacke (1975), in R package 'truncnorm' (Mersmann et al. 2018).

For the putative species within *P. carpi* (see Results), no reliable diagnostic morphometric characters could be found either in the literature (see Haacke 1975) or in the various specimens inspected. However, iris colouration and sexual dichromatism of gular patches were immediately flagged as potential fixed diagnostic fixed differences, and were inspected in 63 photographed individuals from various gene-sequenced populations (Appendix 4: Table S 4). Iris colouration was qualitatively compared using photographs taken by the same camera, flash, and lenses (see Field Sampling, above), and the same colour balance settings. Colours were descriptively assigned. The presence or absence of a clear yellow gular patch was recorded, with absences in several females.

Principle component analyses and group comparisons

Principle component analyses (PCAs) were used to analyse the bioacoustic and morphological data (each separately), to maximise the inter-sample variability of the relevant character traits. These were implemented using function 'prcomp' in R. To avoid over-fitting and redundancy in metrics, only six bioacoustic metrics which encompassed most of the differences observed among locations or populations, were included in the PCA: 1) n notes, 2) upper dominant frequency, 3) note repetition rate, 4) inter-note interval range, 5) note 1 duration deviance, and 6) median call density. For the morphometric analysis, only the *P. garrulus* group putative species with strong support from phylogenetics were represented. Only five morphological characters were included (see above): 1) IN/INSBB, 2) RB/RH, 3) INSH/NB, 4) MBSR and 5) IOS. All variables were scaled to unit variance, centred, and rotated to cause maximum variance.

An analysis of variance (ANOVA) using function 'aov' in R was used to test for a significant difference among the putative species groups, in the principle components with eigenvalues > 1. A Tukey post-hoc test was performed on the ANOVA results using function 'TukeyHSD' in R, to identify significant differences between all putative species pairs.

Calls data: testing for stabilising selection and reproductive character displacement with MRMs

The relationship between call and genetic divergence, and how this relationship is affected by stabilising selection, allopatry, or sympatry of divergent species, was estimated using multiple

regression on matric models (MRMs). Call divergence, the response variable, was presented as a distance matrix of summed, scaled differences between samples, of the principle components with eigenvectors >1 from the call PCA. The absolute difference in, e.g., PC1 between all sample pairs, was scaled between 0 (minimum difference) and 1 (maximum difference), and added to the same scaled differences in, e.g., PC2, each multiplied by the relevant eigenvalues, thus scaling the differences according to the relative amount of call variation explained by each principal component. This gave a total scaled, proportional metric of "call divergence", across all principle components >1. Models including this scaled difference were tested against models using e.g., only PC1 or only PC2, and the scaled difference MRMs returned substantially lower AIC-value for the latter models, indicating a better fit to the data of the scaled metric.

Distance table matrices (among relevant samples) for all explanatory variables were generated in R (4.1.0, R Core Team 2021), using packages ape (Paradis and Schliep 2019) and seqinr (Charif and Lobry 2007) for genetic divergence. Not all recorded individuals were gene-sequenced, in which case another sequenced individual from the same location (when identity was certain) was used to relate the calls and genetics.

Two explanatory variables were fitted to call divergence: sequence divergence (p-distance in *ND2* + *16S*) and co-occurrence pattern as a dummy variable. The latter was coded as 0 = (samples are from the) same clade, 1 = allopatric clades, 2 = parapatric, and 3 = sympatric. Thus, a positive relationship between call divergence and p-distance would indicate a divergence in the calls (phenotype) with genetic divergence (genotype). A positive relationship between call divergence and the dummy variable of occurrence pattern, would support both stabilising selection of call traits within a clade (0, least divergence in calls expected), and reproductive character displacement. The latter would present as selection for divergent call traits between clades in geographic contact (sympatric occurrence, or "in full contact" – 3 – would cause greatest divergence; parapatric occurrence or "in partial contact" – 2 – would cause less divergence; allopatric occurrence – 1 – would have no additional effect).

The full model (both explanatory variables) and models with just one of the explanatory variables, were fitted and compared using model AIC. The R package 'relaimpo' (Grömping 2006) was used to calculate the total percentage explained by the AIC-best model and by each explanatory variable, as an average of two alternative but similar R^2 metrics (x 100) developed for generalised linear models (Genizi 1993, Zuber and Strimmer 2011).

Results

Phylogenetic species delimitation

The species delimitation analysis using the automatic barcode gap detection (ABGD) identified a stable six groups or putative taxa in the initial partitions, henceforth "ABGD clades", resulting in a barcode gap distance (inter vs. intra p-distance) of 9.1%, based on the concatenated mitochondrial dataset (Fig. 4; see Appendix 4: Fig. S 10). These groupings were congruent with the "Chapter 2 clades" except for the merging of two sister clades: *P. g. garrulus* and *P. g. m.* South (Fig. 4). In the recursive partition of the ABGD analysis, up to 28 groups were identified, with a few groupings that were largely incongruent with the total-evidence phylogeny topology (see Appendix 4: List S 1).

The bGMYC analysis identified 24 clusters (putative species) at a threshold probability of $P > 0.95$, 22 clusters at $P > 0.9$, and 12 clusters at $P > 0.5$ (Appendix 4: Fig. S 11). Only the most conservative of these estimates (12 clusters) was included as a comparable putative number of species to ABGD and Chapter 2 clades, henceforth "bGMYC clades" (Fig. 4).

Spatial interpretation of the phylogenetic results supports the species-level split of all seven Chapter 2 clades and of the bGMYC clades *P. g. garrulus* A, B, and C, and *P. carpi* A and B (Table 7; Fig. 4). The MRMs also provided strong evidence for ecological speciation among the Chapter 2 clades (see Chapter 2; Table 7 for *P. g. m.* South vs. *P. g. garrulus*) and speciation by vicariance between bGMYC clades *P. g. garrulus* A & B vs. C, and bGMYC clades *P. carpi* A vs. B (see also Chapter 2). Some net divergence estimates of *16S* (Table 7) are low as a result of the correction for the intraclade divergences, which reduces the value for the net mean inter-clade divergence estimates despite high levels of divergence among individual samples.

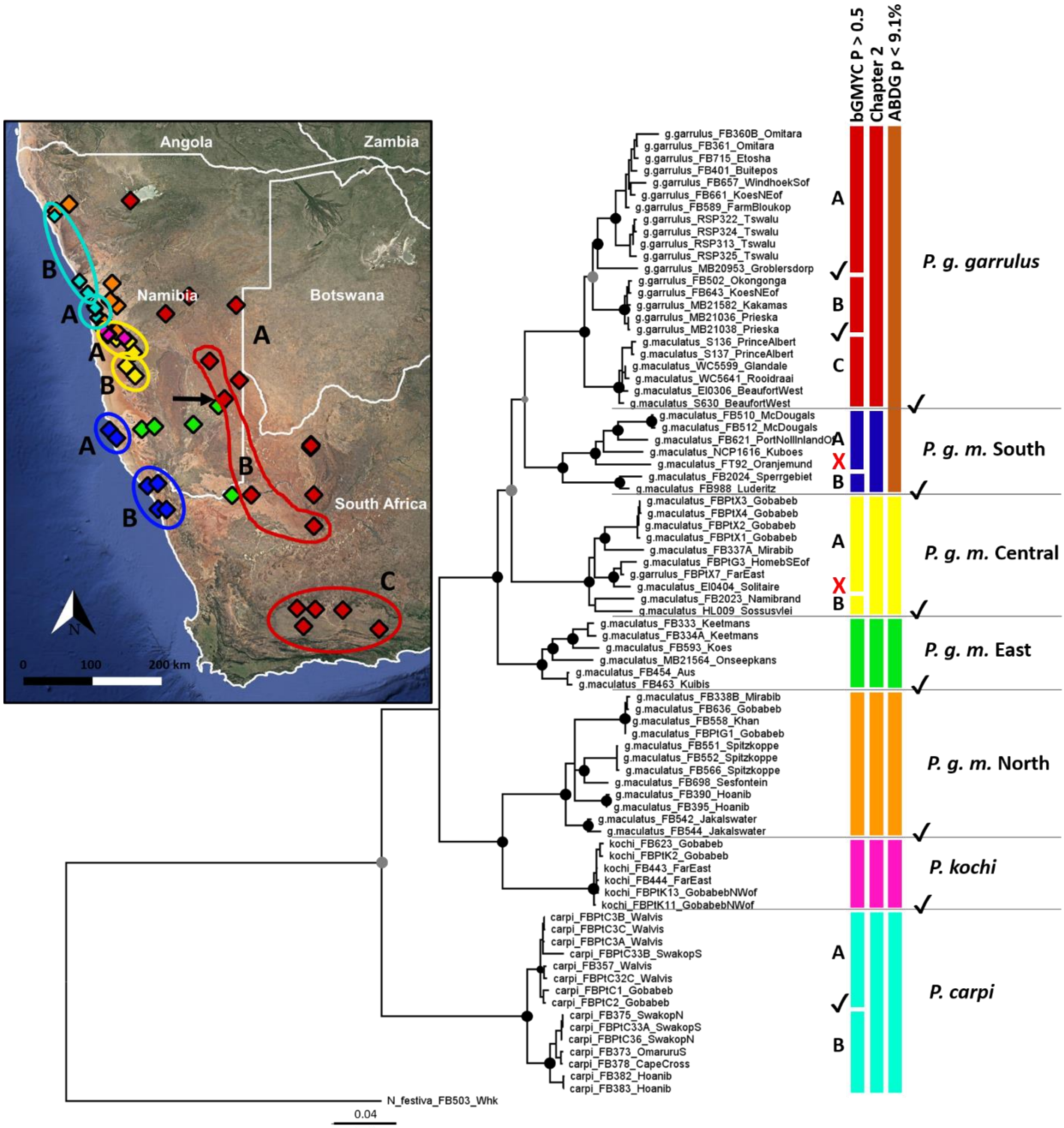

Figure 4: Bayesian topology of *Ptenopus* with clade names from Chapter 2 on the right. Bars show putative species based on two delimitation analyses and Chapter 2, the colours corresponding to sample locations or groups on the map. bGMYC clades within a Chapter 2 clade indicated as A or B on the bars and the map, and by coloured lines. The black arrow on the map indicates a syntopic occurrence of bGMYC clades *P. g. garrulus* A and B. Nodes supported by both maximum likelihood (≥70% bootstrap) and Bayesian (≥0.95 posterior probability) analyses are denoted with black circles and by Bayesian only, with grey circles (but not near branch tips). Nearly supported nodes are similarly shown using smaller circles. Species splits supported by MRMs (Table 7) are shown by a tick-mark, those not supported, by a red 'X'.

Table 7: Summary of phylogenetic-spatial support for splits between sister putative species (clades are dash-separated) based on MRMs from Chapter 2, with (mitochondrial) p-distance as the response variable. Alternative explanatory variable(s) to geographic distance (Alt. separation) and the % variation in p-distance explained by this variable(s) (Alt. var.), vs. explained by geographic distance (Geo var.), are compared, with putative species splits supported (support = yes) if geographic distance is a poor explanatory variable (poor fit to IBD model). Between-group mean p-distance is also indicated for mitochondrial *16S* and *ND2* and nuclear *c-mos* genes.

Clades compared	**Alt. separation**	**Alt. var.**	**Geo var.**	**support**	***16S***	***ND2***	***c-mos***
P. g. garrulus – *P. g. m.* South	climate: coast dist. climate: rain season	18% 55%	7%	**yes**	5.0%	8.2%	0.05%
P. g. garrulus A – B	–	–	9%	**yes**	2.1%	5.2%	0%
P. g. garrulus A&B – C	barrier: scarp	31%	11%	**yes**	1.4%	5.7%	0.17%
P. g. m. South A – B	barrier: Orange R	16%	70%	**no**	2.7%	7.1%	0%
P. g. m. Central A – B	–	–	73%	**no**	1.9%	0.6%	-
P. carpi A – B	barrier: Swakop R	42%	7%	**yes**	0.1%	4.6%	0%

Bioacoustics: mate recognition and reproductive character displacement

Only the first two principal components had eigenvalues >1, and together explained 71% of the variation in the call data (Table 8). The number of notes, speed and rhythmic components of the call contributed substantially (higher loadings) to the variation of both PC1 and PC2, while dominant frequency only contributed substantially to PC1 (Table 8).

ANOVA results showed that both PC1 and PC2 were significantly different among the putative species, and post-hoc comparisons indicate significant differences between most pairs of the putative species, with highly significant differences between all pairs of sympatric putative species. The PCA bi-plot shows very little to no overlap among most Chapter 2 clades, but generally more overlap between bGMYC clades *P. carpi* A and B (Fig. 5).

The MRMs showed a strong, significant positive relationship between genetic divergence and advertisement call divergence, with p-distance explaining 31.2% of call divergence in the AIC-best model (Table 11). Additionally, these models supported a strong, significant relationship between call divergence and occurrence pattern, with variable occurrence explaining 15.2% of call divergence in the AIC-best model (Table 11). This means that individuals from the same putative species display the lowest between-sample call divergence given sequence divergence, while allopatric putative species display higher, parapatric species even higher, and sympatric species the highest divergence in call traits, given sequence divergence between individuals.

Table 8: Rotated component matrix indicating variable loadings and eigenvalues of the six principal components, from the PCA based on six call variables of *Ptenopus* (see bioacoustic analyses).

	PC1	PC2	PC3	PC4	PC5	PC6
1. n notes	-0.13	0.73	-0.16	0.03	-0.07	-0.65
2. upp. dom. freq.	0.49	-0.06	0.32	-0.72	-0.28	-0.25
3. note rate	-0.32	0.53	0.42	-0.35	0.23	0.51
4. int. note int. range	0.35	0.28	-0.75	-0.25	-0.05	0.41
5. note 1 dur. dev.	-0.45	-0.32	-0.33	-0.53	0.49	-0.26
6. call density	-0.56	-0.10	-0.13	-0.16	-0.79	0.10
Eigenvalue	1.62	1.28	0.94	0.69	0.47	0.40

Table 9: ANOVA results comparing principal component scores of PC1 and PC2 across the seven Chapter 2 clades and the two bGMYC clades within *P. carpi*

PC	DF	Sum Sq.	Mean Sq.	F	P
PC1	7	219.91	31.42	87.06	<0.001
PC2	7	136.28	19.469	86.18	<0.001

Table 10: Tukey post-hoc test results comparing principal components PC1 and PC2, between all Chapter 2 clade pairs and the two bGMYC clades within *P. carpi*; additional t-test comparisons between closely related putative sister-species at the bottom. Significance level of ≤0.1 Indicated as '.', ≤0.05 as '*', ≤0.01 as '**', ≤0.001 as '***', and non-significant P-values indicated as-is. The recorded (co-) occurrence pattern between the paired clades is indicated as A = allopatric, S = sympatric, and P = parapatric.

Clades compared	**PC1 sig.**	**PC2 sig.**	**Co-occurrence**
P. carpi B – *P. carpi* A	0.309	0.999	P
P. g. garrulus – *P. carpi* A	0.615	0.072	A
P. g. m. Central – *P. carpi* A	0.703	***	P
P. g. m. East – *P. carpi* A	**	***	A
P. g. m. North – *P. carpi* A	***	***	S
P. g. m. South – *P. carpi* A	0.982	*	A
P. kochi – *P. carpi* A	***	***	P
P. g. garrulus – *P. carpi* B	***	***	A
P. g. m. Central – *P. carpi* B	***	***	A
P. g. m. East – *P. carpi* B	***	***	A
P. g. m. North – *P. carpi* B	***	***	S
P. g. m. South – *P. carpi* B	0.860	***	A
P. kochi – *P. carpi* B	***	***	P
P. g. m. Central – *P. g. garrulus*	1.000	***	A
P. g. m. East – *P. g. garrulus*	**	***	P
P. g. m. North – *P. g. garrulus*	***	***	A
P. g. m. South – *P. g. garrulus*	*	0.734	A
P. kochi – *P. g. garrulus*	***	***	A

P. g. m. East – *P. g. m.* Central	*	1.000	A
P. g. m. North – *P. g. m.* Central	***	0.839	S
P. g. m. South – *P. g. m.* Central	.	0.443	A
P. kochi – *P. g. m.* Central	***	***	S
P. g. m. North – *P. g. m.* East	***	0.782	A
P. g. m. South – *P. g. m.* East	***	0.396	A
P. kochi – *P. g. m.* East	***	***	A
P. g. m. South – *P. g. m.* North	***	0.897	A
P. kochi – *P. g. m.* North	0.180	***	P
P. kochi – *P. g. m.* South	***	***	A

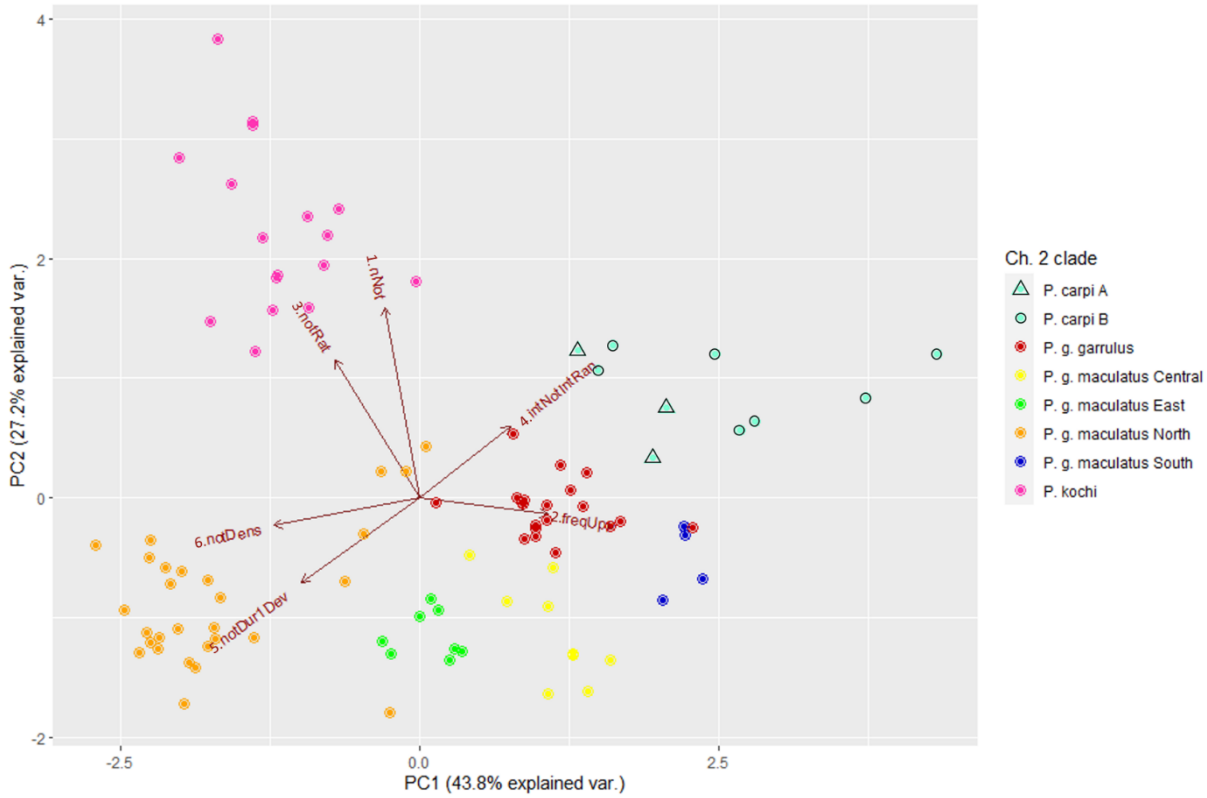

Figure 5: Bi-plot of principal component (PC) scores for PC1 plotted against PC2 from the PCA of advertisement call characters in *Ptenopus* (see Table 7). The Chapter 2 clades, and the bGMYC clades *P. carpi* A and B, are indicated in the legend. The colours match Fig. 4.

Table 11: Multiple regression on distance matrix (MRM) model selection, with scaled call divergence as response variable, and p-distance and occurrence (as same clade/allopatric/parapatric/sympatric) between Chapter 2 clades, as explanatory variables (X). Models are ranked, with model AIC, percentage variation explained by the model (Total var.) and by each variable (Var.), and the p-value (P) for the relationship(s) reported.

Model No.	AIC	Total var.	X	Var.	P
1	1212	46.5%	p-distance	31.2%	<0.001
			occurrence	15.2%	<0.001
2	1575	42.1%	p-distance	42.1%	<0.001
3	1050	27.5%	occurrence	27.5%	<0.001

Morphology

Only the first two principal components had eigenvalues >1, and together explained 69.6% of the variation in the morphometric data (Table 12). The characters RB/RH, INSH/NB, and MBSR contributed more substantially (higher loadings) to the variation in PC1, while IN/INSBB and IOS contributed more substantially to PC2 (Table 12).

ANOVA results showed that both PC1 and PC2 were significantly different among the putative species clades (Table 13), and post-hoc comparisons indicate significant differences between about half of the putative species pairs (as Chapter 2 clades) currently classified as *P. garrulus* (Table 14). The PCA bi-plot shows substantial overlap among most of these putative species (Fig. 6).

The variation of sexual dichromatism and iris colouration from across the range of *P. carpi* (Appendix 4: Table S 4) revealed no females with gular patches in bGMYC clade *P. carpi* B (n = 13 photographed, additional individuals observed), while all females in bGMYC clade *P. carpi* A had gular patches (n = 15 photographed, additional individuals observed); all clade A individuals had red light red to dark red irises (n = 33 photographed, additional individuals observed), while all clade B individuals had silver to brown irises (n = 26 photographed, additional individuals observed). Two individuals photographed south of the Swakop River (in the range of *P. carpi* A) had brown eyes, but one of these was gene-sequenced and turned out to be from clade *P. carpi* B (see Appendix 2: Fig. S 8).

Table 12: Rotated component matrix indicating variable loadings and eigenvalues of the five principal components, from the PCA based on five morphometric characters (see bioacoustic analyses) of *Ptenopus garrulus*-group clades.

	PC1	PC2	PC3	PC4	PC5
1. IN/INSBB	-0.27	0.73	-0.31	0.41	-0.37
2. RB/RH	0.47	-0.11	-0.85	0.06	0.21
3. INSH/NB	0.49	-0.28	0.27	0.69	-0.37
4. MBSR	-0.52	-0.27	-0.07	0.59	0.55
5. IOS	-0.44	-0.55	-0.33	-0.11	-0.62
Eigenvalue	1.51	1.10	0.80	0.76	0.55

Table 13: ANOVA results comparing principal component scores of PC1 and PC2 across the five Chapter 2 clades included in the species *P. garrulus*

PC	DF	Sum Sq.	Mean Sq.	F	P
PC1	4	53	13.249	13.47	<0.001
PC2	4	53	13.249	13.47	<0.001

Table 14: Tukey post-hoc test results comparing principal components PC1 and PC2 between all five *P. garrulus*-group Chapter 2 clade pairs. Significance level of ≤0.1 Indicated as '.', ≤0.05 as '*', ≤0.01 as '**', ≤0.001 as '***', and non-significant P-values indicated as-is. The (co-) occurrence pattern between the paired clades is indicated as A = allopatric, S = sympatric, and P = parapatric.

Clades compared	PC1	PC2	Co-occurrence
P. g. m. Central – *P. g. garrulus*	***	***	A
P. g. m. East – *P. g. garrulus*	0.348	0.348	P
P. g. m. North – *P. g. garrulus*	***	***	A
P. g. m. South – *P. g. garrulus*	1.000	1.000	A
P. g. m. East – *P. g. m*. Central	*	*	A/P?
P. g. m. North – *P. g. m*. Central	0.999	0.999	P
P. g. m. South – *P. g. m*. Central	***	***	A
P. g. m. North – *P. g. m*. East	*	*	A
P. g. m. South – *P. g. m*. East	0.500	0.500	A?
P. g. m. South – *P. g. m*. North	***	***	A

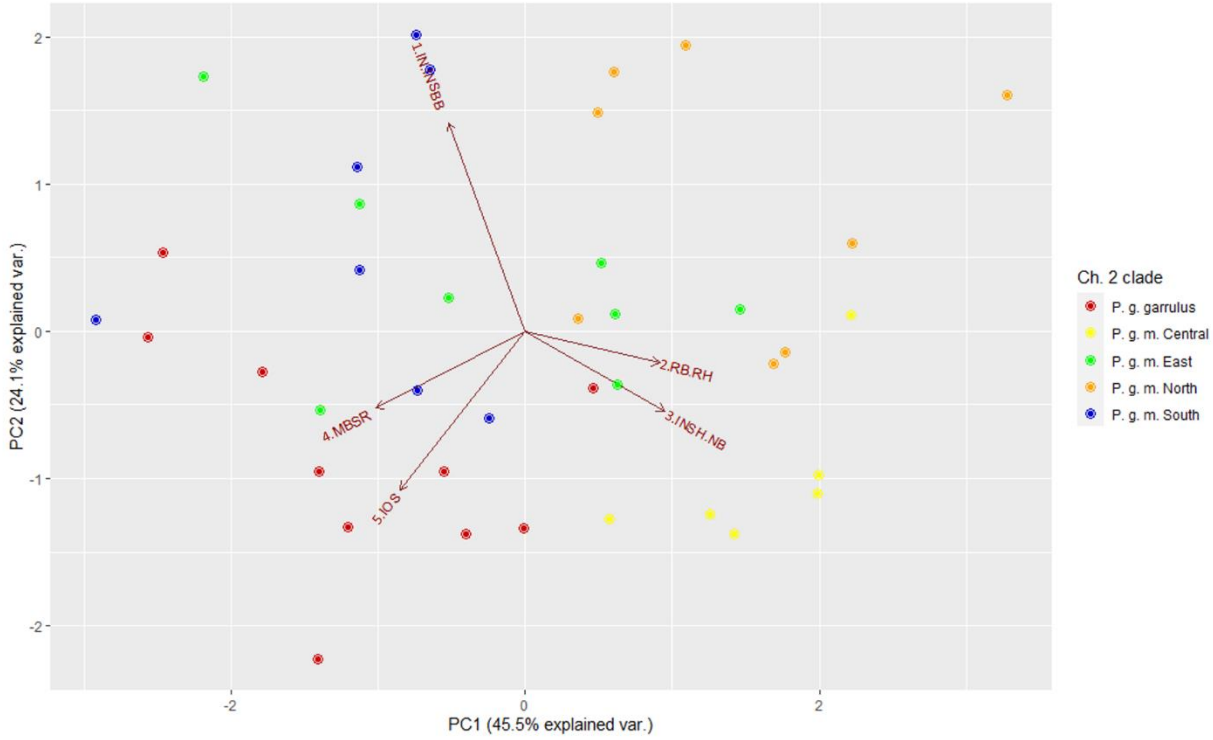

Figure 6: Bi-plot of principal component (PC) scores for PC1 plotted against PC2 from the PCA of morphometric characters in *Ptenopus garrulus*-group species (see Table 12). The Chapter 2 clades are indicated in the legend, and colours match Fig. 4.

Discussion

My results provide multiple lines of evidence to suggest that, according to the general lineage or unified species concept (GLC: de Queiroz 1998, 2007), there are at least eight species within the genus *Ptenopus*. These major clades identified in Chapter 2 (Chapter 2 clades), were generally congruent with species delimitation analyses, although ABGD lumped two of the sister clades (*P. g. garrulus* and *P. g. m.* South), while bGMYC suggested several additional splits within these clades ("bGMYC clades"). I evaluated the various splits, considering only the splits supported by at least three lines of evidence or operative criteria, as valid species.

Genetic-spatial evidence from multiple regression on matric models (MRMs) strongly supported the Chapter 2 clades and some bGMYC clades, as separately evolving genetic lineages (Table 7; Fig. 4). By contrast, the MRMs found significant IBD between the putative bGMYC sister species *P. g. m.* Central A and B, and *P. g. m.* South A and B. However, there were large geographic distances and high levels of divergence between the closest samples of some of these unsupported bGMYC clades. Higher resolution geographic sampling may yet reveal geographic partitioning of these unsupported putative species splits.

The PCA on advertisement calls supported the likely role of these traits in mate recognition among the putative species. The first two principal components (PC1 and PC2) explained most of the variation (71%) in the data and were used as the standard measures of "call difference" in subsequent analyses throughout. The rhythmic or time-related call traits contributed substantial variance to both PC1 and PC2, while dominant frequency only contributed substantially to PC1 (Table 8). This is contrary to the case for many other vocal taxa, where dominant frequency is often the primary species-distinguishing call trait (e.g., Loftus-Hills and Littlejohn 1992, Hobel and Gerhardt 2003). There were substantial and significant differences in the call traits between most putative species pairs, and between all sympatric putative species (Table 10; see Fig. 5). This constitutes strong evidence for the evolution of a vocal mate recognition system in *Ptenopus*, similar to that recorded for other vocal groups such as anurans (Blair 1974, Köhler et al. 2017).

Morphological characters were less reliable than calls for distinguishing the putative species, indicating morphological conservatism in the genus. Although species *P. kochi* and *P. carpi* are clearly distinct (Haacke 1975), the group of putative species within *P. garrulus* are not. The proportions of scales on the nose, IOS, and MBSR, showed promise for distinguishing some *P. garrulus*-group species. The post-hoc tests on the morphology PCA indicated significant differences between over half of these putative species-pairs (in PC1 and PC2 – see Table 14). This conservatism is probably why the taxonomy of *Ptenopus garrulus*-group species has remained stable for nearly a century.

Some diagnostic differences were also identified between the two putative species (bGMYC clades) within *P. carpi*, but these were colour-related traits and will not be identifiable in preserved specimens. Several of the putative species suggested by the bGMYC species delimitation could not be morphologically compared. A morphological study including a large sample size and specimens from all the newly suggested putative species may identify further differences. Overall, only one or two diagnostic features could be found to distinguish the species recognised in this chapter.

The well-supported (by genetic-spatial evidence) bGMYC clade *P. g. garrulus* C is separated from the others by the Great Escarpment, while A and B occurred parapatrically and even syntopically at one site, with no visible boundary or habitat difference separating the clades (see Table 7; Appendix 2: Fig. S 2). The genetic-spatial pattern between clades *P. g. garrulus* A and B could be explained by mitochondrial introgression or interbreeding between previously isolated clades. The bGMYC clade *P. g. garrulus* C showed moderate levels of divergence in the mitochondrial and the nuclear gene *c-mos* (Table 7), likely representing a speciation by vicariance caused by the Great Escarpment (see Table 7; Appendix 2: Fig. S 2). However, no strong evidence supporting an alternative ecological niche for this clade was found. No meaningful comparison of the calls or morphology was possible for these three clade splits, because I had only collected call recordings from clade A, a few gene-sequenced specimens from clade B, and no gene-sequenced specimens from clade C. Thus, due to a lack of additional lines of evidence, I do not recommend bGMYC clades *P. g. garrulus* A, B, or C as valid species pending further investigation.

The (phylogenetic-spatially) supported bGMYC clades *P. carpi* A and B were split across the Swakop River, with clade A to the south and B to the north (Fig. 1; see Appendix 2: Fig. S 8). The ephemeral Swakop River does not currently present a boundary to dispersal, as evidenced by one clade B gecko found south of the river and several geckos observed in the river. This split probably represents a past vicariance event, possibly from when the river flow had been more permanent in the past, e.g. during the Pleistocene (see Korn and Martin 1957). The maintained separation between these two clades despite the lack of a physical barrier is surprising. The advertisement calls were not significantly different between these clades when accounting for experiment-wide error in the post-hoc tests (Table 10), but PC1 (from the call PCA) was significantly different between these clades in an isolated t-test. Although call differences between these clades are not “on par” with other interspecific differences, it is still possible that the difference may be sufficient for the two clades to distinguish one another – possibly using a trait not measured in the PCA. These two clades do differ significantly in one trait, being the (upper) dominant frequency. Alternatively, the split between these two clades may be too recent to have resulted in sufficient call divergence. A larger sample-size of call recordings may settle this uncertainty.

While call-based mate recognition may not be present between the bGMYC clades *P. carpi* A and B, there were two clear diagnostic fixed differences between them. The northern clade B had sexual dichromatism typical of the genus, with only males possessing yellow gular patches, whereas in clade A, both sexes exhibited this trait (see Appendix 4: Table S 4). This difference was observed nowhere else in the genus, and is almost certainly related to mate recognition. Iris colouration also differed between the clades: red eyes in clade A and brown or silver eyes in clade B, with the populations just north of the Swakop River tending to have silver eyes (see Appendix 4: Table S 4). Two geckos found south of the river had brown eyes, and one of them, when sequenced, was found to be part of the northern clade (the other remains unsequenced). I interpret the gular patch and iris colouration traits as diagnostic, fixed differences between these clades. I also interpret the gular patch as a likely mate recognition trait. These traits may have evolved recently as a result of sexual selection acting on sexual dichromatism and/or eye colour. Therefore, based on evidence for evolutionary independence from three operational criteria (phylogenetics, recognition, and diagnosable fixed difference), the bGMYC clades *P. carpi* A and B were accepted as valid species.

There was clear evidence supporting both stabilising selection and reproductive character displacement in the advertisement calls among the Chapter 2 clades. In addition to significant differences among putative species (Table 10), the MRMs indicated a significant positive relationship between sequence divergence and divergence in advertisement call characters (Table 11). This is congruent with theory that signal phenotype generally diverges along with genotype, even by genetic drift (Lande 1981). The significant positive relationship between call divergence and occurrence pattern (Table 11) strongly supports the model of stabilising selection within a species, and character displacement between sympatric species, or (to a lesser degree) species in marginal geographic contact. The AIC-best model included both genetic divergence and occurrence pattern as explanatory variables, with genetic divergence explaining 31.2%, and occurrence 15.2% of call divergence. Simply explained, sympatric clades showed considerably higher call divergence, and parapatric (or “in marginal contact”) clades moderately higher call divergence, given the level of sequence divergence, compared to allopatric clades. While this pattern needs to be further investigated through behavioural experiments on mate choice, this evidence preliminarily supports the reinforcement hypothesis. Reproductive character displacement can evolve due to competition for signal space, rather than reinforcement (Hoskin and Higgie 2010). However, the *Ptenopus* calls differ primarily in rhythmic call traits, not dominant frequency, and in fact frequency differences among sympatric species were usually relatively minor. The patterns of call divergence recorded here are thus best explained by reinforcement, which suggests that advertisement calls are instrumental in maintaining reproductive isolation among *Ptenopus* species.

Strong evidence for ecological niche divergence, in terms of substrate specialisation, supports the remaining putative species identified in Chapter 2 as evolutionary distinct lineages (see Chapter 2). The Chapter 2 clades *P. g. garrulus* and *P. g. m.* South, which were lumped together by the ABGD analysis (Fig. 4), were not distinct in substrate, but occupied distinctive climatic zones (Table 7) and appear to be separated by large tracts of unsuitable substrate. The cumulative evidence from multiple operational criteria (phylogenetic, ecological, recognition, and morphological diagnostic difference or phenetic cluster) strongly supports the recognition of eight distinct species within *Ptenopus*: clades *P. kochi, P. carpi* A, *P. carpi* B, *P. g. garrulus, P. g. m.* South, *P. g. m.* North, *P. g. m.* Central, and *P. g. m.* East.

Interspecific divergence (as uncorrected p-distance) among most of these species (see Chapter 2: Tables 2 – 4) were comparable to described inter-specific divergence in other gecko genera including *Afroedura* (e.g., Busschau et al. 2019, Branch et al. 2021), *Lygodactylus* (e.g., Castiglia and Annesi 2011), *Cyrtopodion* (e.g., Agarwal et al. 2014), *Rhoptropus* (e.g., Lamb and Bauer 2001) and *Pachydactylus* (e.g., Bauer et al. 2006, Heinicke et al. 2011). Due to the method for calculating net mean between-group p-distance in MEGA-X (Kumar et al. 2016), the divergence estimates for species with high intra-clade divergence were likely under-represented. For instance, p-distance between *P. g. m.* South and East was moderately low (8% in *ND2*, 3.5% in *16S*), much lower than the lowest p-distances between samples of the two clades (> 12% in *ND2* and > 6.5% in *16S*), including (interclade) samples that were only 100 km apart compared to several hundreds of kilometres (200 – 500 km) between samples within each clade The interspecific p-distance between *P. carpi* A and B was much lower than among other *Ptenopus* species and was unusually low for the *16S* gene (0.1% net mean in *16S*, although around >0.5% between most sample pairs between the two groups; 4.6% in *ND2*). The unusually low divergence in *16S* compared to *ND2* among *P. carpi* individuals cannot be explained by sequencing errors or missing data, and appears to be genuine. However, the *16S* gene has been show to be unreliable in phylogenetic estimation with variable mutation rates and as such, may be bias in this case as has been found for other taxa (e.g. see Duchêne et al. 2011, Havird and Santos 2014, Chan et al. 2022). Notably, the divergence *patterns* between *P. carpi* A and B across geographic space were similar for both *16S* and *ND2*, and the *ND2* divergence was comparable to minimum interspecific divergence in *Cnemaspis* (e.g., Cyriac 2020, Sayyed et al. 2020).

Conclusions

Operating under the GLC, I present strong evidence from at least three operational criteria (including phylogenetic, ecological, recognition, and diagnostic fixed difference or phenetic cluster)

for the evolutionary independence of eight species within *Ptenopus.* Several additional, plausible species-splits were identified by species delimitation analyses, but their validity could not be sufficiently assessed with my data.

I present clear evidence for a call-based mate recognition system in *Ptenopus*, with evidence for stabilising selection within, and reproductive character displacement among sympatric *Ptenopus* species. The application of MRMs to explicitly test for reproductive character displacement in this way, is relatively novel. According to these findings, advertisement calls likely played a key role in driving or facilitating the speciation process in this genus.

The evidence for reproductive character displacement in *Ptenopus* represents a fairly unique finding. Only one other study was found that investigated reproductive character displacement in gecko advertisement calls, but found evidence to the contrary: a breakdown of species recognition traits and hybridisation occurring between sympatric sister species (Jono 2016). This study is the only recorded case of vocal reproductive character displacement in a non-avian reptile.

CHAPTER 4: A taxonomic revision of the barking geckos (*Ptenopus*: Gekkonidae) with the description of four new species

Abstract

Barking geckos, *Ptenopus* Gray, 1886 are burrowing geckos restricted to the xeric regions of southern Africa. They possess unique vocal abilities, with males producing loud advertisement calls to attract females. The taxonomy of the genus has remained stable for nearly five decades, with three recognised species: *P. carpi* Brain, 1962, *P. garrulus* (Smith, 1849), and *P. kochi* Haacke, 1964. Within *P. garrulus* two subspecies are recognised, the typical form and *P. g. maculatus* Gray, 1866. An integrated taxonomic reassessment of the genus using phylogenetics, ecology, calls, and morphology (Chapter 3) identified the presence of at least eight species within *Ptenopus*. Based on these findings, I elevate the two subspecies *P. g. garrulus* and *P. g. maculatus* to full species, thereby restricting the geographic range of *P. garrulus s.s.* to the greater Kalahari and surrounding areas. Within *P. maculatus,* I recognise and name three new species: *P.* SP1 from the Namaqualand-Richtersveld steppe, *P.* SP2 from the central Namib Desert, and *P.* SP3 from southern Namibia and adjacent Northern Cape of South Africa. In the process, I restrict the range of *P. maculatus s. s.* to the central and northern Namib Desert. Furthermore, I recognise one new species within *P. carpi* and name it *P.* SP4 from the northern coastal Namib Desert, thereby restricting the range of *P. carpi s. s.* to the central Namib Desert, south of the Swakop River. The Namib Desert is the centre of diversity for the genus *Ptenopus*, containing seven of the eight species. Keys to the morphological and vocal diagnostic characters are provided.

Introduction

The barking geckos, genus *Ptenopus*, are endemic to xeric southern Africa. They possess unique vocal abilities, with males producing loud advertisement calls from the entrance of their burrows to attract females and maintain territories (Haacke 1975, Hibbitts 2006). These calls can be heard from well over 100 m away, and in some situations, thousands of males can be heard calling in chorus. The complexity of their burrows are also unique among the southern African geckos, and most of their lives are spent in or around the burrows (Haacke 1975, Polakow 1997, Hibbitts 2006). There are currently three species of *Ptenopus* described: *P. carpi* Brain, 1962, *P. kochi* Haacke, 1964,

and *P. garrulus* (Smith, 1849), the latter containing two subspecies: *P. g. garrulus* and *P. g. maculatus* Gray, 1866. The species *P. garrulus* was initially described under the genus *Stenodactylus,* while *P. maculatus* is the type species of the genus. Fitzsimmons (1935) later synonymised the name *P. maculatus* with *P. garrulus* due to morphological similarity*,* describing the two forms as the subspecies *P. g. garrulus* and *P. g. maculatus*. Despite several taxonomic revisions including specimens from a wide geographic range (Loveridge 1947, Haacke 1964, 1975), this taxonomic model of *P. garrulus* has persisted for nearly 90 years, due to a lack of any substantial morphological variation. The only diagnostic morphological variation between the subspecies that were identified by these authors, were the number of scales around the midbody and across the dorsal or ventral surfaces of the head. These characters were also found to vary within *P. g. maculatus*, to a lesser degree, among specimens from geographically disparate locations (Haacke 1975).

A comprehensive species delimitation process based on phylogenetics, ecology, calls, and morphology (Chapter 3) suggested the presence of eight species within *Ptenopus*. According to these findings, there are two species contained within *P. carpi*: clades *P. carpi* A and *P. carpi* B (Chapter 3). The subspecies *P. g. garrulus* was found to be a distinct species, and the subspecies *P. g. maculatus* was found to contain four distinct species: clades *P. g. maculatus* North, Central, South, and East. *Ptenopus kochi* was found to represent a full species. In this chapter, I take the opportunity to describe four of these clades (*P. carpi* B, *P. g. m.* Central, *P. g. m.* South, and *P. g. m.* East) as new species. In accordance with the findings of Chapter 3, I also elevate *P. g. garrulus* and *P. g. maculatus* to full species, thereby restricting the range of both *P. garrulus s. s.* and *P. g. maculatus s. s.* I further explore the morphological and call characters suggested by Chapter 3 and incorporate them into updated dichotomous keys for diagnosing the eight species of *Ptenopus* according to calls and morphology.

Methods

Systematics and species concept

Preliminary names were assigned to species in the genus *Ptenopus* based on the clades that were identified as species in Chapter 3, under the general lineage species or unified species concept (GLC: de Queiroz 1998, 2007). These species were delineated based on evidence for evolutionary independence from at least three lines of evidence (including phylogenetic, ecological, recognition, and morphological as phenetic cluster or as diagnostic, fixed difference). The original species descriptions, type specimens and type localities were consulted to draw accurate nomenclatural conclusions in this regard, as explained in the species descriptions. Chresonomies for previously

recognised species can be found in Uetz et al. (2022) and Haacke (1975), but I only refer to synonomy of substantial taxonomic revisions and/or the first-use of a name in the taxonomic literature, in each species account. Museum abbreviations used for catalogue numbers: The Natural History Museum (formerly British Museum of Natural History; BMNH); National Museum of Namibia (NMNW); Ditsong National Museum of Natural History (formerly the Transvaal Museum; TM).

Mapping

Updated occurrence maps were produced for *Ptenopus* species based on published records and maps reviewed in Haacke (1975), my own records and observations (Appendix 3 and 5; additional records not tabled) and occurrence records downloaded from GBIF (GBIF.org 2021a, 2021b, 2021c). Positive occurrence of a species was confirmed or inferred based on my own gene-sequenced records and call recordings, while the GBIF downloads and previously published records were only used to infer the presence or absence of the genus in an area, or of a likely species occurrence, compared to my own data. Distributions were then inferred based known habitat determinants of each species (Chapter 2; Chapter 3), considering ecoregion or bioclimate and soils. Soil maps from the Harmonised World Soil Database (Fischer et al. 2008) and the Atlas of Namibia (Atlas of Namibia Team 2022b), and ecoregion or bioclimate maps (Sayre et al. 2013, Dinerstein et al. 2017) were used to for these distributional inferences. Distributions were not modelled, but polygons were manually drawn based on these available sources.

Morphology

Due to the morphological similarity of several species identified in this manuscript, only a few key morphometric measurements and discrete diagnostic characteristics were reported. Morphological characters identifying the eight species were drawn from previous literature (Haacke 1975) and from Chapter 3. The morphological characters distinguishing the species previously referred to as *P. garrulus* in Chapter 3, were compared as box and whisker plots using R (R Core Team 2021), based on the 39 specimens examined (Appendix 4: Table S 3) and the additional extracted data from Haacke (1975 – see Chapter 3). These analyses included the following characters: the number of scales in contact in a straight line across the top of the head between the eyes, in line with the mid-pupil of the pupils – interorbital scales (IOS); the number of midbody scale rows (MBSR); internarial distance, from the nearest edges of the nares, divided by the horizontal breadth of the base of the largest inter-nasal scale in contact with the rostral (IN/INSBB); vertical height of the largest inter-nasal scale, divided by the nostril horizontal diameter or breadth

(INSH/NB); rostral (horizontal) breadth at the lower edge of the lip, divided by rostral (vertical) height (RB/RH). The two diagnostic traits used to distinguish the two species previously referred to as *P. carpi*, were based on 62 photographed specimens from gene-sequenced populations (Appendix 4: Table S 4). Several additional characters were assessed in a qualitative manner, based on all additional inspected specimens (Appendix 5: Table S 5). These included colouration, snout-vent length (SVL) and tail length (TL) in mm, and a descriptive assessment of toe fringes. The mean and range, in parentheses or square brackets (if the phrase is already encapsulated in parentheses), is generally indicated for morphological characters or measurements in the text body.

The following additional measurements were reported for the newly assigned type series' (all measurements in mm): head length (HL), measured ventrally from the tip of the snout to the anterior narrowest portion of the throat, usually between two subtle neck folds (jawline is usually obscured); head width (HW), at the widest point; head height (HH), at the jaw angle behind the eye; horizontal eye diameter (EYE); distance between the anterior corners of the eyes or eye-distance (EED); number of upper labials (UL); number of lower labials (LL); number of post-mental chin scales (PM).

Advertisement calls

The bioacoustic call characters analysed in Chapter 3, based on 839 recorded calls from 98 individuals (Appendix 3: Table S 2), were presented as box and whisker plots and qualitatively inspected to identify key features of non-overlap or very little overlap among species. All additional measured call characters were reported in a separate table, per species (Appendix 5: Table S 6). The bioacoustic call characters included: number of notes (n notes); note repetition rate (s^{-1}); median note duration (ms); note 1 duration deviance (difference between the first note duration and the median note duration, in percentage of the median); median inter-note interval (ms); inter-note interval range (as a percentage of the median inter-note interval: a measure of variability); median call density (median note duration divided by median inter-note interval, no units, with "denser" calls having longer notes and shorter intervals); call duration (s); upper and lower (where relevant) dominant frequency (kHz): most *Ptenopus* species have two frequency peaks of similar amplitude, hence both are termed 'dominant', although the upper peak (or amplitude peak of higher frequency) of the two is usually louder, especially at closer recording distances; basal frequency (Hz); bandwidth, or upper and lower 90% (of peak amplitude) bandwidth frequencies (kHz); call period (call duration plus following inter-call interval).

In the text body, the mean and range are generally reported for all bioacoustic characters with the range in parentheses (or square brackets if the phrase is already encapsulated in

parentheses). Waveform and spectrogram plots were produced using package seewave (Sueur et al. 2008) in R.

Results

Systematics and distributions

Based on the results from Chapter 3 and assessment of the type localities of *P. garrulus* (Smith, 1849) and *P. maculatus* Gray, 1866 (see species descriptions), preliminary species names were assigned or equated to various Chapter 3 clades (Table 15). Inferred species distributions showed a high number of species occurring in western Namibia, while *P. garrulus* occurs across most of the generic range including in Botswana, South Africa, and Zimbabwe, and two additional species occur in the northwestern portions of South Africa.

Table 15: Species names assigned to clades identified in Chapter 3, within the genus *Ptenopus*

Chapter 3 clade	**species name assigned**
P. g. garrulus	*P. garrulus*
P. g. m. North	*P. maculatus*
P. kochi	*P. kochi*
P. g. m. Central	*P.* SP2
P. g. m. South	*P.* SP1
P. g. m. East	*P.* SP3
P. carpi A	*P. carpi*
P. carpi B	*P.* SP4

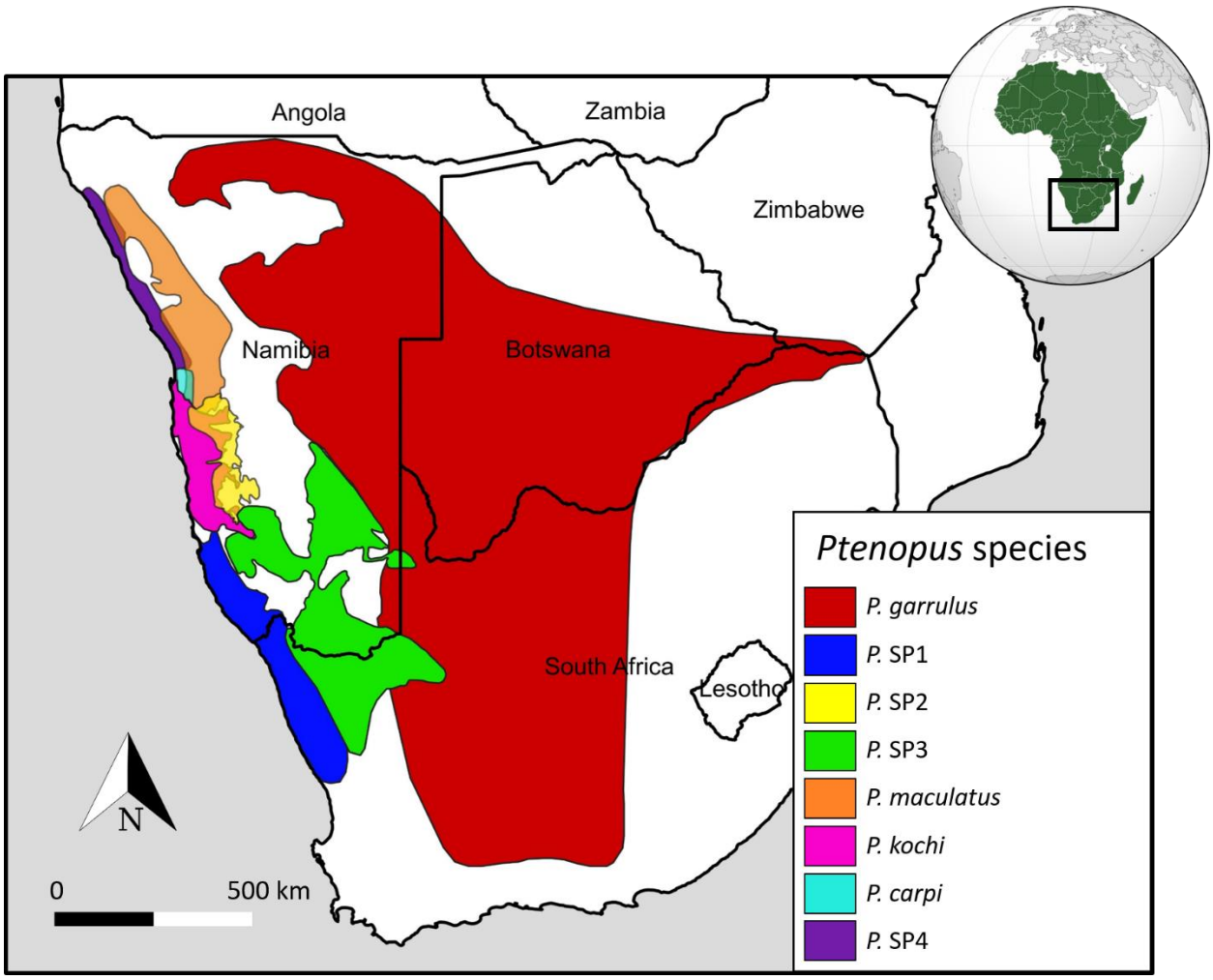

Figure 7: Inferred distributions of *Ptenopus* species in Africa.

Morphology

Among the species previously included within *P. garrulus* (Table 15)*, P. maculatus* and *P.* SP2 had lower MBSR than other species, with some overlap (Fig. 8: A). Species *P. maculatus* exhibited lower, but overlapping interorbital scale counts than other species (Fig. 8: B). *Ptenopus* SP2 had a broader internasal scale base vs. internarial distance (IN/INSG) compared to other species, and non-overlapping with *P.* SP3 and *P.* SP1 (Fig. 8: C). Most species overlapped substantially in internasal scale height compared to nostril breadth (INSH/NB), although *P.* SP3 had a substantially smaller or less high internasal scale than *P.* SP2 (Fig. 8: D). *Ptenopus* SP1 have substantially narrower and taller rostral scale than *P.* SP2, while this trait (GB/RH) overlapped among other species (Fig. 8: E).

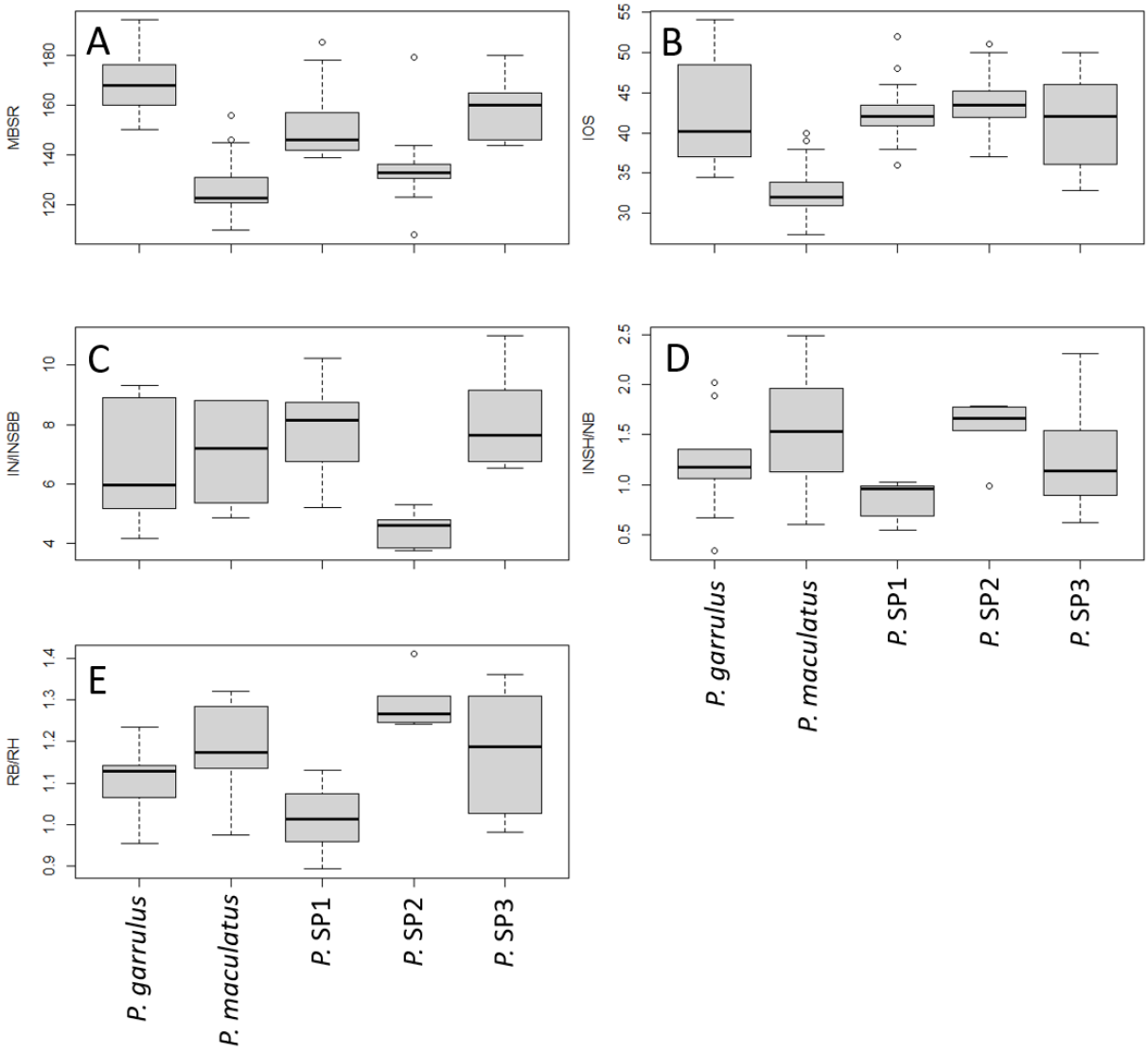

Figure 8: Box and whisker plots of morphometric variables for species previously included in *P. garrulus*. A = midbody scale rows; B = interorbital scales; C = internarial space per internasal scale base breadth; D = internasal scale height per nostril breadth; E = rostral breadth per rostral height.

Table 16: Measurements (in mm) and scale counts for the type series of the newly described *Ptenopus* species. Relative snout measurements for selected species (previously included in *P. garrulus*) are also provided.

Catalogue No. →	**NMNW R 11390**	**NMNW R 11391**	**NMNW R 11389**	**NMNW R 10839**	**NMNW R 11394**	**NMNW R 11395**	**NMNW R 13c**	**NMNW R 13h**
Species	*P.* SP1	*P.* SP1	*P.* SP3	*P.* SP3	*P.* SP2	*P.* SP2	*P.* SP4	*P.* SP4
Type status	Holotype	Allotype	Holotype	Allotype	Holotype	Allotype	Holotype	Allotype
Sex	male	female	male	female	male	female	male	female
Snout-vent length	49.94	50.79	51.41	52.18	48.75	46.81	52	51.9
Tail length	35.20	34.38	30.61	NA	35.79	31.08	37.05	37.1
Midbody scale rows	170	171	166	162	129	135	131	131
Inter-orbital scales	51	56	48	43	51	56	48	40
Head length	15.87	15.97	15.97	15.3	14.62	13.68	15.29	15.45
Head width	11.19	11.23	11.15	11.6	10.99	9.97	11.58	11.58
Head height	6.68	6.41	6.85	7.8	5.57	5.05	7.7	7.62
Eye diameter	3.46	3.51	3.46	3.02	3.15	2.93	4.49	4.22
Eye-distance	5.89	6.00	5.93	5.7	5.81	5.49	5.69	5.9
Postmental scales	13	9	6	6	6	7	5	4
Upper labials	8	8	6	8	8	8	9	9
Lower labials	8	9	8	6	8	8	9	9
RB/RH	0.76	1.16	1.42	1.24	1.44	1.31		
IN/INSBB	6.90	5.90	8.71	7.54	4.69	3.96		
INSH/NB	0.50	0.79	1.03	0.86	1.09	1.38		

Advertisement calls

The calls of *P. kochi*, *P. carpi* and *P.* SP4 had more notes than other species (Fig. 9: A). Species *P. garrulus, P. maculatus* and *P. kochi* had higher note repetition rate than other species (Fig. 9: B). Note 1 duration deviance was substantially higher in *P. maculatus* than all other species, and moderately higher in *P.* SP3 (Fig. 9: C). Inter-note interval was substantially longer in *P. carpi* and *P.* SP4, and moderately longer in *P.* SP1 than in other species (Fig. 9 D). Median call density was notably higher in *P.* SP3, *P. maculatus* and *P. kochi* than in other species (Fig. 9: E). The (upper) dominant frequency was substantially different between the two closely related species *P. carpi* and *P.* SP4 (Fig. 9: F).

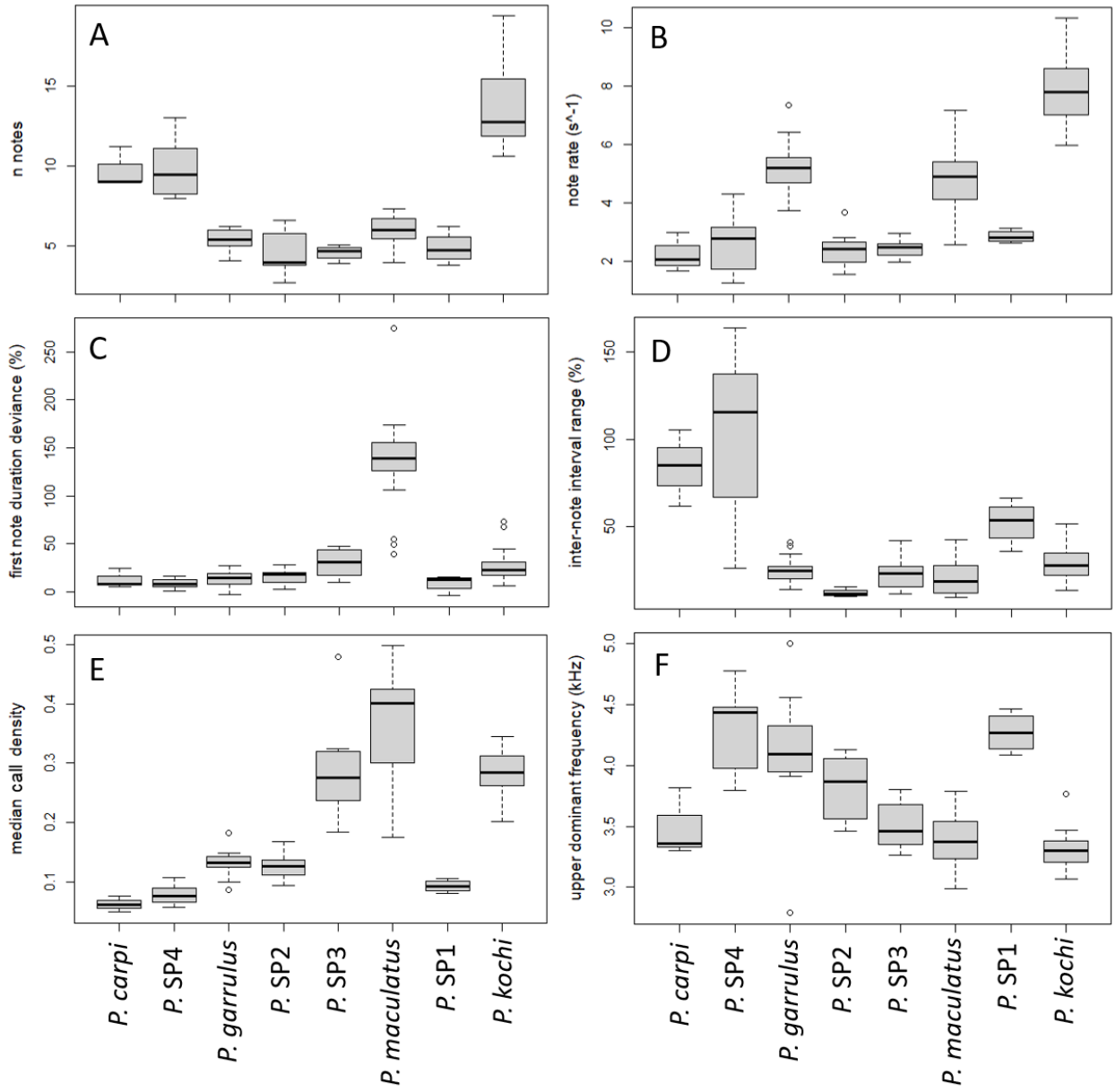

Figure 9: Box and whisker plots of diagnostic advertisement call characters for *Ptenopus* species.

Species descriptions and revisions

Disclaimer:

All species names provided in this thesis would be considered *nomen nudum* or taxonomically invalid under the ICZN Code (see Article 9). However, to avoid future confusion, I have provided only numbered placeholder names for the new species. As such, no etymology sections are provided. Similarly, all taxonomic adjustments made in this thesis will only be considered valid once published in a peer-reviewed journal. Furthermore, all type specimens assigned in this thesis are not taxonomically valid types, and allotypes are assigned as equivalent to (preliminary) paratypes.

Reptilia: Squamata: Gekkonidae

Ptenopus garrulus (Smith 1849)

Kalahari barking gecko

Afrikaans: Kalahari blafgeitjie / Kalahari grondgeitjie

Figs. 10–11

Synonyms. *Stenodactylus garrulus* – A. Smith, 1849. III. Zool. S. Afr. Rept., Append.: 6.

Ptenopus garrulus (part) – Boulenger, 1885: 15.

Ptenopus garrulus garrulus – FitzSimons, 1935: 524.

Ptenopus garrulus maculatus (in part) – Branch 1998: 265.

Note. The types Sir Andrew Smith did not specify the type locality of his collections beyond "sandy districts in the interior of Southern Africa" (Smith 1849). He described type specimens as buff orange, having a spotted colour pattern and poorly or non-barred tail. Sir Andrew travelled and collected extensively in Namaqualand and briefly in southern Namibia, prior to his description of *P. garrulus* (Skelton 2018). These areas contain the three species referred to in Chapter 3 as clades *P. g. m.* South (herein *P.* SP1), *P. g. m.* East (herein *P.* SP3), and clade *P. g. garrulus* (Chapter 3). The last-named species is the only one of the three that occurs on orange, sandy substrate "inland", with the colour to match. "Inland of South Africa" also rules out the coastal regions (and thus *P.* SP1), so the southern Kalahari in the northern or central regions of the Northern Cape Province, South Africa, most likely contain the type locality. I therefore elevate the subspecies *P. g. garrulus* to full species, considering this equivalent to clade *P. g. garrulus* from Chapter 3, and thereby exclude *P. g. maculatus* from *P. garrulus sensu stricto.* This treatment of *P. garrulus s. s.* is equivalent to the subspecies *P. g. garrulus* as it was treated by Haacke (1975), with the addition of specimens from the

northern portions of the Western Cape (e.g. near Beaufort West), South Africa, that were referred to as *P. g. maculatus* by Branch (1998) and subsequent authors.

Due to this revision, I restrict the range of *P. garrulus s. s.* to the greater Kalahari and surrounding areas including Namibia (excluding the Namib Desert), most of Botswana, the northern and central portions of South Africa, and extreme southern Zimbabwe (Fig. 7). The later-assigned common name "common barking gecko" (e.g., Branch 1998) is probably not best-suited for this species, since it referred to a now defunct taxonomic grouping that occupied the entire generic range. Although it is still a widespread species, "Kalahari barking gecko" is suggested as more appropriate.

Types. BMNH 1946.8.23.43–49, collected from "sandy districts in the interior of Southern Africa", by Andrew Smith prior to 1849. FitzSimons (1937b) noted these specimens as the types used by Smith.

Diagnosis. A small *Ptenopus* (SVL max 53.4 mm, mean 46.6 mm, n = 22) with a moderate tail (TL 69% [range 50 – 86] of SVL, n = 17) and a generally slender appearance. This species is distinguished from all other species in the genus by a combination of the following morphological characters: toes extensively fringed laterally (similar to *P. kochi* vs. weakly fringed in *P. carpi* and *P.* SP4, and intermediately fringed in other species), with fringe length equal to the breadth of the toe between fringes (vs. fringe length generally less than half toe breadth in *P. carpi* and *P.* SP4, and about half in other species); having white pigmented ventral scales on ventral surface except for pink, unpigmented scales on the (hand/foot) palms/soles (vs. pink, unpigmented patches on the tail and limbs in *P. kochi,* dark grey or brown speckling on the palms/soles of *P.* SP3, and entirely white palms/soles and verntum in other species); having usually MBSR 150 – 194, mean 169 (n = 31) (substantially overlapping with *P.* SP3 and *P.* SP1, vs. usually < 140 in *P.* SP2 and < 150 in *P. maculatus*, and < 135 in *P. carpi* and *P.* SP4); more-or less speckled appearance dorsally (similar to *P. kochi*, vs. banded pattern in *P. carpi* and *P.* SP4 and usually large paired, light, ovoid markings interspaced by dark mottled patches in other species). It is further distinct from *P. carpi* and *P.* SP4 by the nasals being more swollen and the nostrils partially covered by internal projections of the upper labials; from *P. kochi* by having fingers laterally fringed with pointed triangular scales (vs. elongated pointed scales in *P. kochi*).

Its advertisement call is distinguished from that of other congeners mainly by a combination of the following characters (see Fig. 9): 5 notes (range 4 – 6) (similar to other species, vs. usually > 7 notes in *P. kochi*, *P. carpi* and *P.* SP4); notes uttered at rapid and regular rate (note repetition rate 5.28 s^{-1} [range 3.76 – 7.37], similar to *P. maculatus,* vs. faster in *P. kochi* and generally < 3.7 in other species; inter-note interval range 25.25% [range 14.11 – 34.63], vs. > 36% in *P.* SP1 and usually >

60% in *P. carpi* and *P.* SP4); shortest note duration of all species (22 ms [range 16 – 31]); lower median call density (0.13 [range 0.09 – 0.18]) than *P. maculatus, P. kochi,* and *P.* SP3; higher (upper) dominant frequency (3.49 kHz [range 3.30 – 3.82]) than other species except *P.* SP4 and *P.* SP1

Colouration. In life (Fig. 10), colouration varies with substrate colour, as in other species. The colour pattern tends to be speckled or juxtaposed with light, dark, and coloured scales, the lighter (cream or white) speckles usually tending to larger dots or asymmetrical bars in some locations. On the tail, light-and-dark bars are usually evident dorsally. Background colour forms include brick red in the Kalahari sands, white to cream or grey in white sand, and grey-brown. Ventrally, animals are immaculate white, with faded dark grey or brown patterning around the lateral margins, and pinkish patches on the (hand/foot) palms/soles. Males have bright yellow, heart-shaped gular patches which may extend onto the lateral margins of the head and body, and anterior surfaces of the legs.

In preservative, the lighter colours fade to off-white or beige, and all the darker colours appear various shades of dark brown or grey. The brighter colours, especially yellow, fade completely.



Figure 10: *Ptenopus garrulus* in life (FB589), from farm Bloukop (-25.0953, 19.8497, 1064 m), Namibia. Photo by Francois S. Becker.

Advertisement call. The advertisement call (Fig. 11) consists of 5.4 notes (4 – 6) uttered in rapid succession, with a note rate of 5.28 s^{-1} (range 3.76 – 7.37). Note duration is short, 22 ms (range 16 – 31), and highly regular, with note 1 deviance -3 – 27%. Inter-note intervals are short (168 ms [range 114 – 234]) and regular, although latter intervals can be slightly longer; inter-note interval range 14 – 34%. Call density relatively low (0.13 [range 0.09 – 0.18]). Call duration shortest of all species, only 0.86 s (range 0.6 – 1.2). The basal frequency is ~450 Hz (range 373 – 508) but very soft and may be inestimable, with harmonic bands louder towards the dominant frequency at 4.2 kHz

(range 3.9 – 5.1); a very slight lower peak frequency band is evident at around or just below half the dominant frequency (roughly 1.3 – 2.6 kHz, cannot always be reliably estimated). Frequency appears to modulate briefly up, then down by ~100 Hz with each note, or remain constant. The (human) perceived pitch tends to be higher than that of most other species. Bandwidth (90%) is difficult to estimate consistently: approximately 0.6 – 8.2 kHz, usually wider than other species.

During peak chorus activity, this species calls much more frequently than other species, the call period being very short: mean 25 s, and as low as 4 s. However, calling activity is short in most localities visited by the author, usually commencing around 20 min after sunset, and lasting only 20 – 40 mins. Calling may continue later if the moon is up and conditions are warm, but at a severely decreased rate.

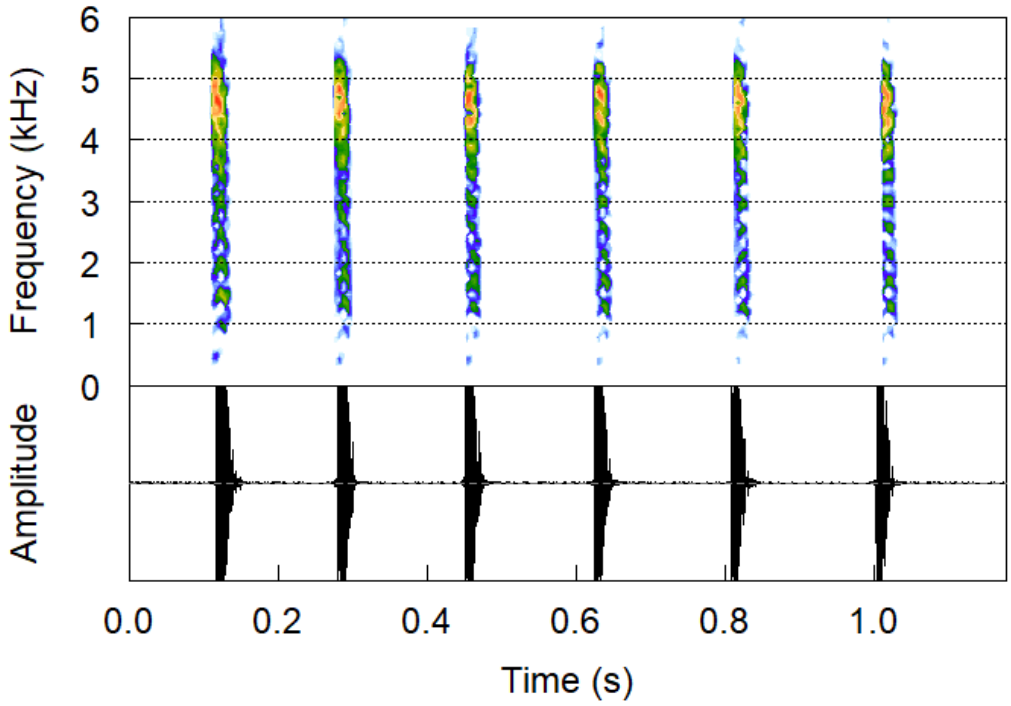

Figure 11: Sonogram (top) and oscillogram (bottom) of a typical advertisement call of *Ptenopus garrulus* (FB1033) from northeast of Koës, Namibia (-25.711, 19.306, 1034 m). In the sonogram, warmer colours (to red) are higher sound energy, cooler colours (to blue) are lower sound energy.

Habitat and distribution. This is the most widespread member of the genus, occurring on sandy soils across much of arid or semi-arid southern Africa, but not in the Namib Desert or the pro-Namib (Fig. 7). It occurs on sandy flats, interdunes, and on vegetated dunes or hummocks, but usually not on steep dunes. In one location (south of Windhoek) it was found occurring on consolidated, silty soils, but this is not usually the case.

Natural history. The breeding season takes place in spring, from September to November in the southern parts of the range, with calling occurring earlier and egg-laying taking place after this

period (Hibbitts et al. 2005, 2012, Hibbitts 2006). In the northern portions the breeding season is earlier, from around July to September. For a more in-depth description of the natural history, see: (Hibbitts and Whiting 2005, Hibbitts et al. 2005, 2007, 2012, Hibbitts 2006).

Ptenopus maculatus Gray, 1866

Damaraland barking gecko

Afrikaans: Damaraland grondgeitjie / blafgeitjie

Figs. 12–13

Synonym. *Ptenopus maculatus* – Gray, 1866. Proc. zool. Soc. Lond. PZSL 1865: 640, pl. 38: 1.

Ptenopus garrulus (part) – Boulenger, 1885: 15.

Ptenopus garrulus maculatus (part) – Mertens, 1955: 51; Haacke, 1975: 221.

Note. *Ptenopus maculatus* was described from "Damaraland", collected by Karl (=Charles) J. Andersson. This species was briefly synonomised under the name *P. garrulus* (Boulenger 1855). Subsequently, the subspecies *P. g. maculatus* was erected (FitzSimons 1935), which had been regarded as valid ever since. However, most of the earliest references to the subspecies *P. g. maculatus* (e.g., FitzSimons 1935, 1937a, Loveridge 1947) did not include specimens from the type locality, but instead referred to *P.* SP1 and *P.* SP3 Mertens (1955), Haacke (1975), and several subsequent authors did refer to specimens from a broader range, including "Damaraland", as *P. g. maculatus.*

According to Andersson's notes and the location names used at the time (see map in Andersson 1872), "Damaraland" referred to the area north and northwest of Windhoek. This area contains specimens from two species: *P. g. garrulus s. s.* and the species identified in Chapter 3 as clade *P. g. m.* North. Although Gray's description of the species was not specific enough to distinguish between the different lineages described here, the type specimens (BMNH 1946.8.23.53-54) and a detailed drawing from the type description, only match the colour pattern and appearance of clade *P. g. m.* North (Chapter 3). I therefore elevate the subspecies *P. g. maculatus* to full species, thereby restricting the range of *P. maculatus sensu stricto* to the central and northern Namib Desert, north of the Kuiseb River (Fig. 7).

Haacke (1969) described the calls of *P. maculatus s. s.* and of *P.* SP2 from areas near Gobabeb (Central Namib Desert, Namibia), but was unable to link specimens with their calls. Both calls are described and distinguished in a standard taxonomic framework in this chapter.

This species has been referred to as the "spotted barking gecko" in online sources (e.g., Uetz et al. 2022), probably in reference to the specific epithet "maculatus", which Gray (1866) used to describe the densely mottled or "blotched" dark patches between the light ovoid markings, even

though he did not draw these markings as spots. However, this name is confusing because a truly spotted colour pattern characterises other congeners (*P. garrulus*, *P. kochi*, and southern populations of *P.* SP1) and not *P. maculatus*. Therefore, "Damaraland Barking gecko" in reference to the type locality and range of this species, is much more appropriate.

Types. BMNH 1946.8.23.53-54, collected from "Damaraland", (currently) Namibia, by Karl (=Charles) J. Andersson prior to 1866.

Diagnosis. A moderately large *Ptenopus* (SVL max 60 mm, mean 51.7 mm, n = 34) with a moderate tail (TL 69% [range 58 – 78] of SVL, mean, n = 21) and stout appearance. Although sister to *P. kochi,* it is easily distinguished from *P. kochi, P. carpi, P.* SP4, and *P. garrulus*, but is morphologically very similar to species *P.* SP2, *P.* SP3, and *P.* SP1 sp. nov (which were previously included in *P. g. maculatus*). It differs from the former (more different-looking) species group by the following characters: toes being intermediately fringed laterally (vs. weakly fringed in *P. carpi* and *P.* SP4*,* and extensively fringed in *P. kochi*), with fringe length being at least half the breadth of the toe between fringes; having white pigmented ventral scales on entire ventral surface (vs. pink, unpigmented patches on the tail and limbs in *P. kochi*, and pink unpigmented scales on the palms/soles of *P. garrulus*); having MBSR 110 – 156, mean 127 (vs. > 180 in *P. kochi* and usually > 160 in *P. garrulus*); by dorsal colour pattern with four to five large, paired, ovoid markings on a darker background, interspaced by dark brown wavy or broken blotches (vs. more finely spotted in *P. garrulus* and *P. kochi,* and banded in *P. carpi* and *P.* SP4).

From the latter group of species, *P. maculatus* is only distinguished by lower MBSR (127 [110 – 145] vs. 178 [151 – 139] in *P.* SP1, 134 [123 – 144] in *P.* SP2, and 158 [144 – 180] in *P.* SP3); lower IOS (38 [32 – 40] vs. 43 [38 – 46] in *P.* SP1, 44 [37 – 50] in *P.* SP2, and 42 [33 – 50] in *P.* SP3, for reference 42 [34 – 54] in *P. garrulus*); from *P.* SP2 by larger adult size (mean 51.7 mm, up to 60.03 mm, vs. mean 48.6 mm, max 54.9 mm in *P.* SP2).

The advertisement call of *P. maculatus* is distinguished from that of all other *Ptenopus* species mainly by a combination of the following characters (see Fig. 9): 6 notes (range 4 – 7) (similar to other species, vs. > 7 notes in *P. kochi*, *P. carpi,* and *P.* SP4); note 1 duration deviance is longer than all other species (usually > 120%, mean 138, max 247%), except in the northernmost near-coastal populations where it can be as low as 40%; highest median call density (0.51 [range 0.18 – 2.98]) of all species. The note rate (4.99 s^{-1} [range 2.58 – 7.17]) is also usually faster than other species except for *P. kochi* and *P. garrulus,* and the upper dominant frequency (3.4 kHz [range 3.0 – 3.8]) is lower than other species except for *P. kochi* (mean 3.3 kHz)*,* and only moderately lower than *P. carpi, P.* SP4 and *P.* SP3 The audible pitch (to humans) of the call is moderate compared to other species, and that of the first note is usually higher than the rest.

Colouration. In life (Fig. 12), the dorsal colour pattern consists of 4 – 6 large, cream/yellow/white paired ovoid markings from the neck to tail base with various light, coloured and dark brown juxtaposed speckles, darker colours coalescing into ill-defined paired patches between the oval markings; white ovoid markings laterally. Background dorsal colour is usually light brown or camel with occasional bright orange, pink, or purple speckling. The ventrum is immaculate white. Males have bright yellow gular patches. In some specimens, the yellow pigment extends onto the nose or patchily onto lateral margins of the head and neck.

In preservative, the lighter colours fade to off-white or beige, and all the darker colours appear various shades of dark brown or grey. The yellow fades completely within hours of fixation in formalin and eventually in ethanol.



Figure 12: *Ptenopus maculatus* in life from Gobabeb Research Station (-23.5610, 15.040, 395 m), Namibia. Photo by Francois S. Becker.

Advertisement call. The advertisement call of *P. maculatus* (Fig. 13) consists of 6 notes (range 4 – 7) uttered in rapid succession, with a note rate of 4.99 s^{-1} (range 2.58 – 7.17). Note duration is long, 50 ms (range 26 – 71), with the first note being notably longer than the rest, note 1 duration deviance 138% (range 39 – 274). The inter-note intervals are usually short (145 ms [range 88 – 303]) and regular with inter-note interval range 20% (range 10 – 42), although the first interval may be slightly longer. Call density is higher than all other species, mean 0.36 (range 0.17 – 0.50). Call duration is short (1.1 s [range 0.8 – 1.4]). The basal frequency is 380 Hz (range 301 – 517) but very soft and may be inestimable, with harmonic bands louder towards the (upper) dominant frequency at 3.4 kHz (range 3.0 – 3.8), while a clear but softer lower frequency peak is evident at around 1.7 kHz (range 1.4 – 2.0), just below half the (upper) dominant frequency. Frequency appears to modulate briefly up (~0.2 kHz), then drastically down by ~0.5 kHz with the first note, and less

severely down in the notes following. The first note tends to be louder, have a slightly higher dominant frequency, and a slightly higher (human) perceived pitch than the following notes. Bandwidth (90%) is difficult to estimate consistently: approximately 0.9 – 4.8 kHz.

Call period (mean 110 s) varies greatly, but can be as low as 10 seconds during peak chorus activity. Calling commences shortly before or at sunset and may continue throughout the night after moonrise and before moonset. Calling activity appears to continue throughout the year, but is sometimes more pronounced around the austral spring and/or summer. Rainy weather usually causes calling activity to cease for days to weeks. This species hardly ever calls during the day.

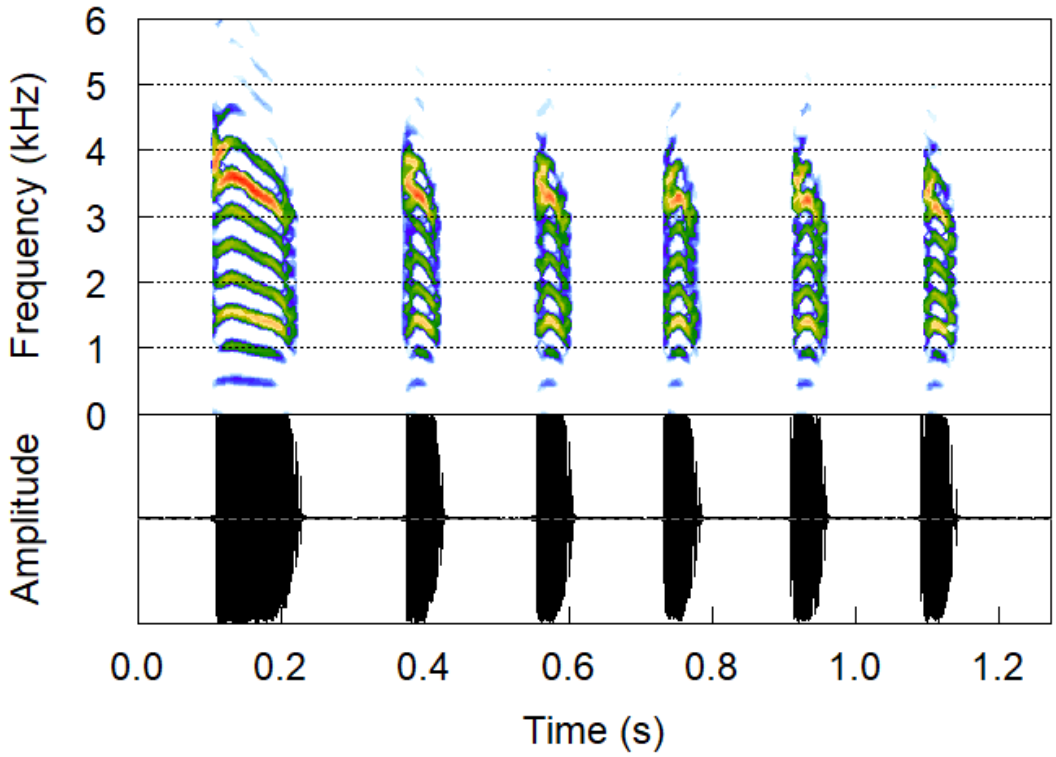

Figure 13: Sonogram (top) and oscillogram (bottom) of a typical advertisement call of *Ptenopus maculatus* (FB830) from Gobabeb Research Station north of the Kuiseb River, Namibia (-23.559, 15.041, 405 m). In the sonogram, warmer colours (to red) are higher sound energy, cooler colours (to blue) are lower sound energy.

Habitat and distribution. *P. maculatus* occurs on hard substrates such as gravel or silt, throughout the central and northern Namib Desert. The central Namib gravel plains are almost entirely granite-based gravel (Atlas of Namibia Team 2022a) and the species is widespread and common here. In the northern reaches of its distribution, *P. maculatus* occurs in patchy floodplains, riverbeds or pans with a high clay or silt content, but do not occupy the rocky areas. It does not appear to occur closer than 25 – 50 km, nor further than 200 km, from the coast. In the west it occurs syntopically with *P. carpi* and *P.* SP4 which prefer the same soils, but occurs closer to the

coast in the fog zone. Along the edges of the Namib Sand Sea, *P. maculatus* occurs sympatrically and even syntopically with *P.* SP2, but there is a separation of preferred substrate: *P. maculatus* is denser in areas around granite outcrops with freshly weathered gravel, whereas *P.* SP2 is denser in sandier patches. On the northern banks of the Kuiseb River in the central Namib Desert, *P. maculatus* occasionally occurs sympatrically with *P. kochi*.

Natural history. Like the other species, *P. maculatus* males call from their burrow entrance and antagonistic behaviours between males are common if two burrow entrances are within ~2 m. Aggressors will sometimes utter the advertisement call as they look for the transgressing male, possibly to elicit a response. Males will increase calling frequency if a female approaches the burrow. Males may exit the burrow and approach the female, and after a brief encounter will follow the females into the burrow. Alternatively, the male will remain in place until the female scratches at the burrow entrance; the male then reverses into the burrow and the female follows. The male will utter a muffled version of the advertisement call inside the burrow, where copulation likely commences. After about an hour, the female will emerge partially from the burrow entrance, surveying the surrounding area with her head erect for a long time. This behaviour is repeated throughout the first night after a female moves into a new burrow. This may be to memorise the location of the new burrow entrance. Males presumably move out of the burrow after the female has moved in, as males and females have not been observed living in the same burrow.

Ptenopus kochi Haacke, 1964

Koch's barking gecko

Afrikaans: Koch se blafgeitjie / duinblafgeitjie

Figs. 14–15

Synonyms. *Ptenopus kochi* – Haacke, 1964. Sci. Pap. Namib Des. Res. Stat. 25: 1, pls. I–V.

Ptenopus garrulus maculatus (in part) – FitzSimons, 1943: 13.

Ptenopus garrulus (in part) – Brain, 1962.

Note. A description of the morphology of this species is not revised here, although some natural history observations and the first formal bioacoustic call description are provided.

Holotype. TM 28809, adult male, collected from Gobabeb, Namibia (-23.62, 15.05, 408 m), by Wolfe D. Haacke at in October 1963.

Paratypes. TM 24993–4, collected by Charles Koch in October 1957; TM 25880–1, 25887, 25889–90, collected by Charles K. Brain in May 1959; TM 28442–6, 28448–55, 28625–7, collected by Wolfe D. Haacke in October 1963. Locality: same as holotype.

Diagnosis. The largest *Ptenopus* (SVL max 65.1, mean 61.1, n = 23) with the longest tail (TL 82.8% [range 62.7 – 96.1] of SVL, n = 23), an overall plump appearance, extensive toe fringes and elongated fringed scales on the fingers compared to other species. It is distinguished from all other congeners by a combination of the following characters: body and head scales finer than other species, with MBSR 187 – 210 (vs. generally < 200 for *P. garrulus* and < 180 for other species); dorsal colour pattern finely speckled with a few somewhat enlarged cream or yellow spots (somewhat similar to *P. garrulus,* vs. banded in *P. carpi* and *P.* SP4 and large paired, oval marking in *P. maculatus, P.* SP2, *P.* SP3, and northern populations of *P.* SP1); the tail, (hand/foot) palms/soles are completely or partially pink and unpigmented, whereas the rest of the ventrum is white (similar to *P. garrulus* except for the tail, vs. immaculate white in *P. maculatus, P.* SP1*, P.* SP2, and black- or dark grey-speckled in *P.* SP3); the yellow pigment in males is not limited to the throat, but extends over the sides of the snout, head, neck, and body. For a more detailed morphological description, see Haacke (1964; 1975).

The advertisement call of *P. kochi* is distinguished from that of all other congeners (see Fig. 9), mainly by a combination of the following characters: 13 notes (range 10 – 19) (vs. 9 – 13 notes in *P. carpi* and *P.* SP4 and < 8 notes in all other species) uttered in quicker succession than other species (7.9 s^{-1} [range 6.0 – 10.3]) due to shortest inter-note intervals (98 ms [range 75 – 130] ms); notes and inter-note intervals regular; median call density (0.28 [range 0.20 – 0.34]) higher than most species, but similar to *P.* SP3 and lower than *P. maculatus*. The dominant frequency is generally lower than other species (3.3 kHz [range 3.1 – 3.5]), but the call is generally higher-pitched (human audible) than other species, although similar to allopatric *P. garrulus*, and longer (1.2 – 2.4 s) than other species except for *P. carpi* and *P.* SP4



Figure 14: *Ptenopus kochi* in life (FB2120), from NamibRand Nature Reserve, Namibia (-24.9770, 15.9302, 1143 m). Photo by Francois S. Becker.

Advertisement call. The advertisement call (Fig. 15) consists of 13 notes (range 11 – 19) uttered in rapid succession with a note rate of 7.90 s^{-1} (range 5.98 – 10.33). Note duration is short (27 ms [range 18 – 40]) and relatively regular, sometimes getting slightly shorter as the call progresses, with note 1 duration deviance low (20% [range 6 – 31]). The inter-note intervals are usually short (98 ms [range 75 – 130]) and regular with inter-note interval range low (30% [range 14 – 52]). Median call density is high (0.28 [range 0.20 – 0.34]) and call duration long (1.6 s [range 1.2 – 2.4]). The basal frequency is 382 Hz (range 301 – 492) but very soft and may be inestimable, with harmonic bands louder towards the upper dominant frequencies, the upper at 3.3 kHz (range 3.1 – 3.88) and a clear lower dominant frequency around 1.4 (range 1.1 – 1.9) kHz, about half the upper dominant frequency. Frequency appears to remain constant throughout the notes and call, as is the case with the (human) perceived pitch. Bandwidth (90%) is difficult to estimate consistently: approximately 0.7 – 4.9 kHz.

Call period (mean 74 s) varies greatly, but can be as low as 9 seconds during peak chorus activity. Calling commences shortly before or at sunset, and usually ends at nightfall. Sporadic calls may occasionally be heard later at night or even in the morning. Calling activity may continue throughout the year, but is more pronounced around the austral spring and/or summer. Rainy weather usually causes calling activity to cease for days to weeks. This species hardly ever calls during the day or late at night.

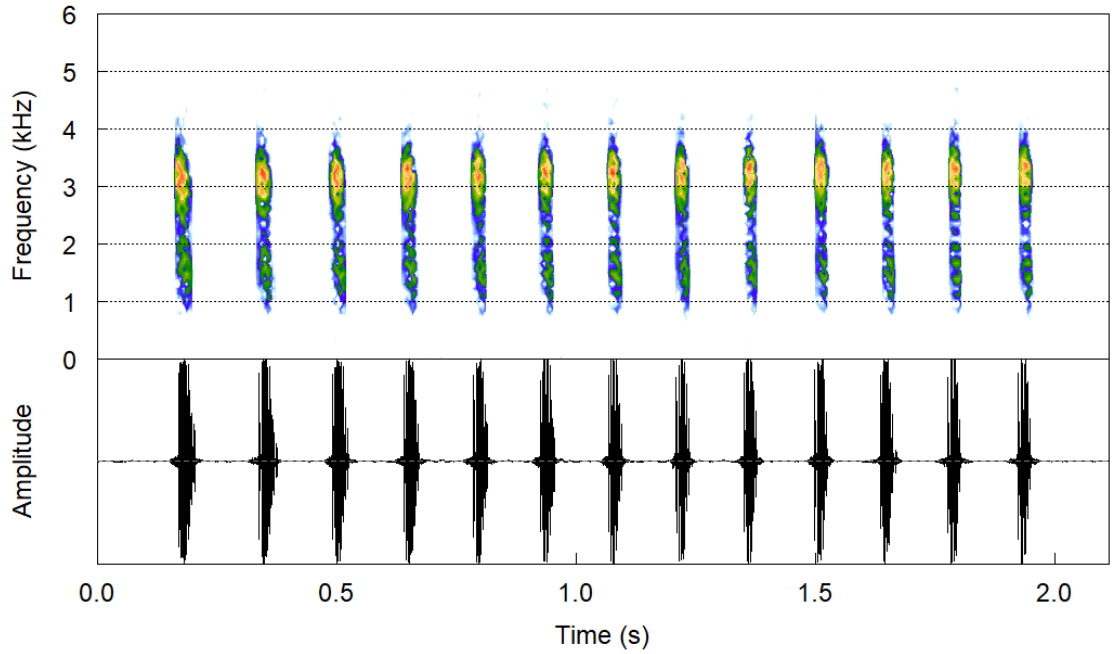

Figure 15: Sonogram (top) and oscillogram (bottom) of a typical advertisement call of *Ptenopus kochi* from Gobabeb Research Station south of the Kuiseb River, Namibia (-23.5660, 15.0372, 402 m). In the sonogram, warmer colours (to red) are higher sound energy, cooler colours (to blue) are lower sound energy.

Distribution and habitat. *Ptenopus kochi* occurs entirely within the Namib Sand Sea in the central Namib Desert, Namibia. The Kuiseb River is the northern border of occurrence although it is also found within and on the northern banks of the Kuiseb. It occurs up to the coast near Walvis Bay in the northwest, although it has not been recorded close to the south-western corner of the Sand Sea near Lüderitz. It is possible that *P. kochi* does not occur in the predominantly winter rainfall zone starting from this area southwards, but it does occur ~80 km inland (predominantly summer rainfall).

The habitat of this species was initially described as the silts in Kuiseb River and the interdune plains by (Haacke 1964), and was even referred to afterwards as the Interdune Barking gecko (Branch 1998). In reality, this species rarely occurs on the interdunes and its occurrence in and around the Kuiseb River is more coincidental with sandy intrusions from the adjacent Namib Sand Sea. Its primary habitat is characterised by the dune plinth, being the base of the dune where the sand is very loose, but before it slopes more steeply. Its occurrence on the interdunes is only peripheral, except in instances where dunes are small, poorly formed, or continuous, resulting in large areas with loose sand similar in composition and slope to a typical dune plinth. True interdune plains in the Sand Sea are occupied primarily, or solely, by *P.* SP2

Some *P. kochi* do occur on the northern banks of the Kuiseb River, occasionally in sympatry with *P. maculatus*, on sandier patches of the gravel plains. These animals are genetically divergent

from those on the southern side of the river (see Chapter 2), and are usually duller in colouration to match the brownish substrate. The typical colour form in the eastern reaches of the sand sea is ochre to orange, matching the sand. It also occurs parapatrically with *P. carpi* along the Kuiseb River.

Natural history. This species calls much more intensively and for a shorter period of time than its sympatric or parapatric congeners (*P. carpi*, *P. maculatus* and *P.* SP2). It also usually calls earlier than congeners, with calling commencing before sunset and ceasing shortly after darkness falls, although a few sporadic calls are sometimes uttered later in the night. It has previously been called nocturnal (Haacke 1969), but is crepuscular. This species is also more frequently observed on the surface during the day (e.g., Murray and Lease 2015) than congeners except perhaps for *P.* SP2, usually in the late afternoons.

Courtship seems to be somewhat variable. Some courtship encounters witnessed were similar to that of *P. maculatus*, with the female simply approaching the calling male and the male responding with a few calls. The female then scratches rapidly at the burrow entrance to indicate she wants to enter, after which the male retreats backwards and the female follows him inside. In other encounters, the males may exit the burrow and display their gular patch to the approaching female. Other variations including mutual head bobbing and soft vocalisations have also been observed (Polakow 1997). The burrows of *P. kochi* tend to be more complex than other species, often with more than one entrance (Polakow 1997; own observations). Males, females, and hatchlings share burrows for extended periods of time. See Polakow (1997) and Haacke (1964; 1978) for more extensive notes on *P. kochi* courtship and ecology.

Ptenopus carpi Brain 1962

Carp's barking gecko

Afrikaans: Carp se blafgeitjie

Figs. 16–17

Synonym. *Ptenopus carpi* – Brain, 1962. Cimbebasia 1: 14, pls 1b, c & e, fig. Ib; Haacke, 1964: 3.

Note. This species was described from the gravel plains near Gobabeb in the central Namib Desert Namibia (probably near -23.480, 14.983, based on the nearest locality to Gobabeb where *P. carpi* currently occurs). The species delimitation analyses in Chapter 3 indicated the presence of two species contained within *P. carpi:* clades *P. carpi* A and B, with clade A only occurring south of the Swakop River and clade B only to the north (except for one individual which was found 2 km south of the river). Only the southern species, clade *P. carpi* A (Chapter 3), occurs at the type locality of *P.*

carpi and is therefore assigned the name *P. carpi sensu stricto,* while clade *P. carpi* B is described as *P.* SP4 in this chapter. I hereby restrict the range of *P. carpi s. s.* to south of the Swakop River. In light of this revision, the advertisement call of *P. carpi s. s.* has not been recorded or formally described; Gramentz (2008) described the call of *P.* SP4

This is the only *Ptenopus* species that lacks sexual dichromatism, with both sexes displaying a yellow gular patch. My observations contradict the original description of *P. carpi* (Brain 1962) which described females, collected in the month of May, as having no gular patch. Haacke (1975), who collected *P. carpi* extensively from around the type locality, made observations corresponding with my own: that all *P. carpi* females possess a yellow gular patch. Haacke surmised that the presence of these patches on females may be seasonal. However, I have collected, photographed and observed multiple *P. carpi s. s.* females from across their range including the type locality, and throughout the year (see Appendix 4: Table S 4; additional observations not tabled), and have not observed a single female *P. carpi s. s.* without a gular patch; nor have I observed a gular patch in any *P.* SP4 females. Since the gular patches fade shortly after specimen preservation (not mentioned by previous authors), I argue that Brain (1962) may have assumed the sexual dichromatism while describing the species based on the preserved specimens, and failed to notice the gular patches on live females. Alternatively, the gular patch may be missing under rare circumstances, although under what circumstances I shall not surmise.

Holotype. TM 25973, adult male, collected "on gravel plain approximately 1 mile north of the [Kuiseb River]" at Gobabeb, Namibia (-23.5, 15.0), by Charles K. Brain in May 1959.

Paratypes. TM 25966–70, 25972, 25974–25979, 25981–86, 25990–93, 25995, 25997–8, 26207 (10 adults, 10 subadults and 6 juveniles), all from the same locality as the holotype.

Diagnosis. A large *Ptenopus* (SVL max 64.7 mm, mean 53.7 mm, n = 33) with a moderate tail (TL 66.7% [range 61 – 72] of SVL) and lean appearance, with slender limbs and reduced toe fringes compared to other species. It is morphologically indistinguishable from *P.* SP4 except by sexual dichromatism, with females having a yellow gular patch as in males (vs. no female gular patch in *P.* SP4); by red or ochre iris colouration (vs. silver to brown in *P.* SP4). It is the only *Ptenopus* (except for *P.* SP4) with a clearly banded dorsal colour pattern on the body and tail. For a more detailed morphological diagnosis and description, see Haacke (1975).

Its advertisement call is distinguished from that of all other *Ptenopus* species mainly by a combination of the following characters (see Fig. 9): 10 notes (range 9 – 12) in the full call (similar to *P.* SP4 [up to 13] vs. 10 – 19 notes in *P. kochi* and <8 notes in all other species); inter-note intervals decrease notably to the middle of the call and increase towards the end (vs. starting short and getting longer in *P.* SP4, notably longest last interval in *P.* SP1, and relatively consistent for all other

species); upper dominant frequency (3.5 kHz [range 3.3 – 3.8]) lower than *P.* SP4 (4.3 kHz [range 3.8 – 4.8]), to which the call is otherwise similar; inter-note interval range (62% [range 62 – 105]) much higher than other species but similar to *P.* SP4; median call density (0.06 [range 0.05 – 0.08]) notably lower than other species and moderately lower than *P.* SP1 (mean 0.092) and *P.* SP4 (mean 0.08); call duration (3.6 s [range 3.3 – 3.8]) is longer than other species except *P.* SP4 (up to 7.2 s); the call is softer, lower-pitched and more monotonous than that of other species (except *P.* SP4 and northern populations of *P.* SP1).



Figure 16: Adult male *Ptenopus carpi* (FBPtC12) in life from Carpi Wash northwest of Gobabeb Research Station, Namibia (-23.4809, 14.9833, 375 m, near the type locality). Photo by Francois S. Becker.

Advertisement call. The advertisement call (Fig. 17) consists of 10 (9 – 12) notes uttered in slow succession, with a note rate of 2.25 s^{-1} (range 1.68 – 3.01). Note duration is short (26 ms [range 22 – 29]) and regular with note 1 duration deviance 12.8% (range 5.5 – 24.8). The inter-note intervals are very long (560 ms [range 323 – 682]) and usually irregular, the inter-note interval range being the highest of any species (84% [range 62 – 105]) except *P.* SP4; intervals are longer at the start and end of the call, shortest in the middle. Median call density is the lowest of all species (0.06 [range 0.05 – 0.08]) and call duration is very long (3.6 s [range 3.3 – 3.8]). The basal frequency is 392 Hz (range 310 – 517) but very soft and usually inestimable, with harmonic bands louder towards the dominant frequencies, with the upper dominant frequency at 3.5 kHz (3.3 – 3.8) and a clear lower dominant frequency (sometimes more emphasised than the upper) around 1.4 kHz (1.1 – 1.9), less than half the upper dominant or frequency. Frequency appears to remain constant throughout the notes and call, as with the (human) perceived pitch, which is low and monotonous compared to most other species. The call is also notably softer than most other species. Bandwidth (90%) is difficult to estimate consistently, but is broad: approximately 2 – 5 kHz.

The call seems to vary more than other species, with calls sometimes having as few as one to three notes, especially late at night. Call period (mean 349 s) varies greatly, but is usually much lower than other species, and has not been recorded any faster than 2 min during peak chorus activity. This species does not chorus as notably as other species. Calling activity is crepuscular to nocturnal, calls starting well after sunset and often continuing throughout the night to some degree. One instance of a notable chorus was recorded near Walvis Bay airport on 19 Apr 2018. Chorusing started abruptly at ~25 min after sunset, and lasted for about 20 minutes, with very reduced calling activity continuing later into the night. Calling appears to be somewhat seasonal with a peak around April to June, but phenology may be different in different locations. Calling activity is more pronounced on warmer evenings, but may continue despite cold, foggy conditions.

As with other species, *P. carpi* calls from the burrow entrance, although it may also call (advertisement call) occasionally while roaming. This could not be observed directly, but a wandering gecko was often spotted in the area where a call was just heard – although wandering geckos are easier to spot than geckos in a burrow.

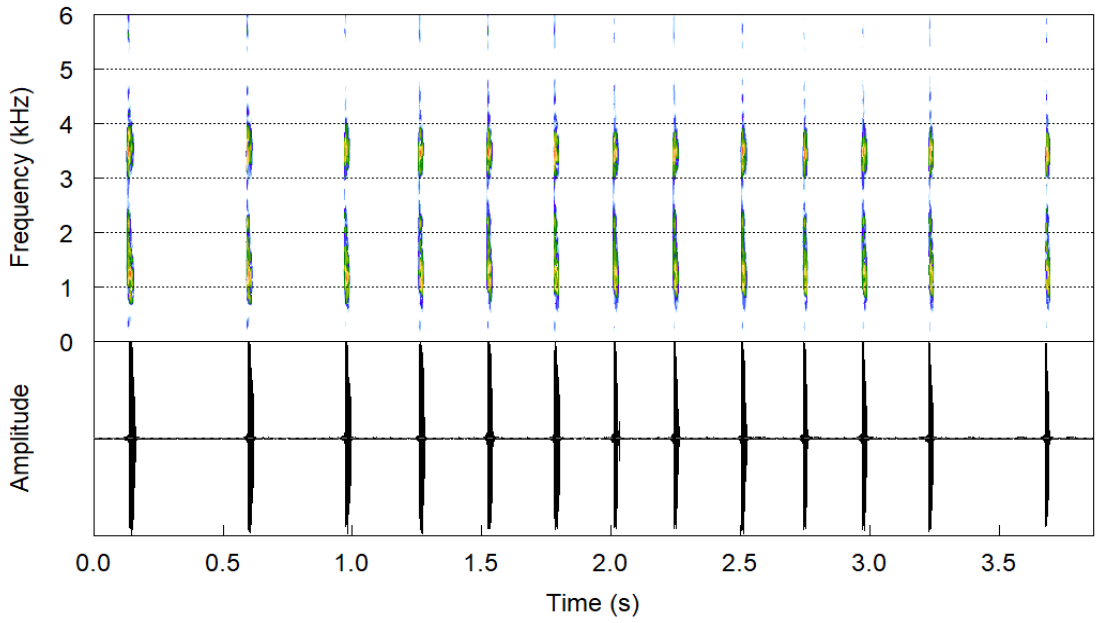

Figure 17: Sonogram (top) and oscillogram (bottom) of a typical advertisement call of *Ptenopus carpi* (FB865) from Carpi Wash near Gobabeb Research Station (-23.4822, 14.9809, 369 m – likely the type locality), Namibia. In the sonogram, warmer colours (to red) are higher sound energy, cooler colours (to blue) are lower sound energy.

Distribution and habitat. *P. carpi* occurs on hard gravel plains in the central Namib Desert, Namibia, from the northern banks of the Kuiseb River to the southern banks of the Swakop River, central Namib Desert (Fig. 7). It does not tolerate soft, sandy substrates. *P. carpi* occurs in the Atlantic fog band, from the high water mark to approximately 50 km inland. It is probably the most common vertebrate on the lichen plains, but becomes increasingly rare when approaching the occasionally grassy plains to the east. Here it occurs syntopically with *P. maculatus*, parapatrically with *P. kochi* and *P.* SP2 along the Kuiseb River (the latter species occurring in or south of the river, while *P. carpi* occurs north of it). *Ptenopus carpi* and *P. kochi* can occasionally be found only metres from each other, on two different substrates.

In light of this revision, *P. carpi* is severely range-restricted, with an estimated extent of occurrence ~1,400 km^2. While most of its habitat is encapsulated by formally protected areas (Namib-Naukluft National Park and Dorob Park), mining or industrial activities and the degradation of the gravel plains by motorists does occur within this range. According to current knowledge, it does not trigger any IUCN Red List threat categories, but it may qualify as Near Threatened. Studies on the population size and stability are needed to comprehensively reassess its' threat-status.

Natural history. *Ptenopus carpi* does not call nearly as often as most congeners (except *P.* SP4), but appears to spend more time wandering on the surface, even far from the burrows. It is active much later into the night than congeners, often only emerging well after dark, and its activity is not dampened by cold or foggy conditions (as in most other species).

The burrow is usually shallower and simpler than those of other species, but may have two to three side-branches and typically has an escape exit (as in other species). These terminate a few millimetres beneath the surface of the soil, usually behind the burrow entrance at an angle. Gravid females observed contain two eggs, which are presumably laid in the burrow, as in other species.

P. carpi utters two different calls: the advertisement call and the single-pulse call, also previously noticed for the closely related *P.* SP4 (Gramentz 2008). Gramentz (from observations in captivity) suggested it may be a threat call, but my observations suggest this is unlikely the function. It was never notably uttered in response to my own movements, and the first individuals to utter this call were often far from me. The single-note call is uttered frequently in the wild by both *P. carpi* (and *P.* SP4), usually late at night after most calling has ceased. Individuals clearly respond to each other, with several answering the first call uttered within a second or two. It is possible that this is a spacing mechanism, or a way for individuals to keep track of the population's general location as they wander on the surface. A squeak may be uttered if the animal is molested (as with other species) but this does not sound the same as the single-note call observed – which is identical to a

single note in the advertisement call. For more details on the natural history of *P. carpi*, see previous works (Brain 1962, Haacke 1975).

Ptenopus SP1

Figs. 18–20

Synonym. *Ptenopus garrulus maculatus* (in part) – FitzSimons, 1935: 525.

Note. Specimens of this species (referred to in Chapter 3 as clade *P. g. m.* South) were treated as *P. g. maculatus*, first by FitzSimons (1935) and then by several subsequent authors, including in some notable morphological comparisons by Haacke (1975). All coastal and near-coastal specimens in the southern Namib Desert (Namibia) and Richtersveld (South Africa) previously referred to as *P. g. maculatus*, are presently assignable to this species. It appears not to occur sympatrically with any other species except potentially on the eastern margins of its range.

Holotype. NMNW R 11391, adult male, collected from Grosse Brucht Bay, south of Lüderitz, Namibia (-26.73379, 15.10412, 29 m), by Francois Becker and Bertha Buiswalelo on 27 September 2022.

Allotype. NMNW R 11391, adult female, with the same collection details as the holotype.

Additional material examined. See Appendix 4: Table S 3 and Appendix 5, Table S 5 for specimens and photographed, non-vouchered material examined (n = 30).

Diagnosis. A small *Ptenopus* (SVL max 45.1 mm, mean 39.4 mm, n = 19) although specimens collected in the north are larger (up to 50.8 mm), with a long tail (TL 77% [range 59 – 99] of SVL) and an overall lean appearance. It is easily distinguished from *P. kochi, P. carpi*, *P.* SP4, and *P. garrulus*, but is morphologically very similar to *P. maculatus, P.* SP3 and *P.* SP2 (previously included together in *P. g. maculatus*). It differs from the former (more different) species group by the following characters: toes being intermediately fringed laterally (vs. weakly fringed in *P. carpi* and *P.* SP4 and extensively fringed in *P. kochi*, and *P. garrulus*), with fringe length being at least half the breadth of the toe between fringes (vs. generally less than half in *P. carpi* and *P.* SP4, generally equal to in *P. kochi* and *P. garrulus*); having white pigmented ventral scales on entire ventral surface (similar to *P. carpi* and *P.* SP4, vs. pink, unpigmented patches on the tail and limbs in *P. kochi*, and pink unpigmented scales on the [hand/foot] palms/soles of *P. garrulus*); having MBSR 141 – 185 (similar to *P. garrulus,* vs. 105 – 131 in *P. carpi* and *P.* SP4 and 187 – 222 in *P. kochi*); by having on the dorsum 4 – 5 paired, more-or-less symmetrical, light-coloured (from yellowish to cream or white) ovoid markings on the body, about half the diameter of the eye or larger, interspaced by dark brown wavy or broken blotches (vs. 5 or more smaller – usually smaller than half eye diameter, variably shaped and often asymmetrical light markings, usually in more than two rows, which tend to dots in

P. kochi and most *P. garrulus* populations, or fuse into jagged lines in some *P. garrulus* populations; light and dark markings fused into mottled bands in *P. carpi* and *P.* SP4). It is further distinct from *P. carpi* and *P.* SP4 by the nasals being more swollen and the nostrils partially covered by internal projections of the upper labials; from *P. kochi* by having fingers laterally fringed with pointed triangular scales (vs. elongated pointed scales in *P. kochi*).

From the latter group of species (previously included in *P. g. maculatus*), *P.* SP1 is only marginally distinct (see Fig. 8) in having MBSR 151 (139 – 185) and IOS 43 (36 – 52) (overlapping with other species vs. usually MBSR 127 [110 – 145] and IOS 33 [27 – 38] in *P. maculatus*). It also has a generally smaller internasal scale than *P.* SP2, with higher IN/INSBB of 7.8 (5.2 – 10.2) and INSH/NB of 0.84 (0.55 – 1.03), lower than most species (vs. 4.5 [3.7 – 5.3] and 1.57 [0.99 – 1.79] in *P.* SP2). It also has a taller or narrower rostral scale than most species, with RB/RH = 1.01 (0.9 – 1.1) (overlapping with other species, vs. 1.29 [1.2 – 1.4] in *P.* SP2). Eye colour at the coast is blue-grey in the north and reddish in the south, and light yellow in the northern inland populations (vs. usually ochre in *P. maculatus* and *P.* SP2 and *P.* SP3).

Its advertisement call is distinguished from that of other *Ptenopus* species mainly by a combination of the following characters (see Fig. 9): 5 notes (range 3 – 6) (similar to other species, vs. > 7 notes in *P. kochi, P. carpi* and *P.* SP4) with consistent duration (vs. notably longer first note in *P. maculatus* and in most *P.* SP3 individuals); inter-note interval range 52% (range 36 – 66), intervals being more variable than other species except *P. carpi* and *P.* SP4, with the last interval notably the longest; median call density lower than other species (0.09 [range 0.08 – 0.11] except for *P. carpi* and *P.* SP4 (0.05 – 0.11). In coastal populations the call tends to be slower, lower-pitched and have fewer notes, whereas inland populations have faster calls.

Holotype description. (Fig. 18). Adult male, SVL 49.94 mm with original tail 35.20 mm (70.5% of SVL). Body and head covered with minute hexagonal to round scales of a similar size, dorsally and ventrally, with scales on limbs notably larger. Small mid-ventral incision for removal of liver sample. Body slender, MBSR 170, IOS 53, HL 15.87 mm, HW 11.19 mm, HH 6.68 mm, EED 5.89 mm. Upper labials 8, lower enlarged labials 8 with an additional small, elongate scale on the inflection of the jaw; eight granules bordering the mental. Clear but not prominent superciliary ridges tapering above mid-eye level; a single row of elongated scales around the anterior and upper margins of the eye with curved tips posteriorly, the scales being smaller and rounder along the posterior and dorsal edges. Prenasal scales swollen, separated by one small, asymmetrical hexagonal internasal scale in contact with the rostral, with IN/INSBB 6.90, INSH/NB 0.50; nostrils partially covered by projection from the upper nasal. Rostral is narrow and tall, with RB/RH 0.76, MB/RB 0.89. Eyes intermediate, EYE 3.46 mm, pupil vertical; ear opening is oblique (~45°), and narrow with small, slightly projecting

scales at the anterior margin. Toes elongate, flattened, with elongate fringed scales, small, pointed, triangular fringes on the fingers; strong nails on fingers and toes, being larger and thicker on the fingers.

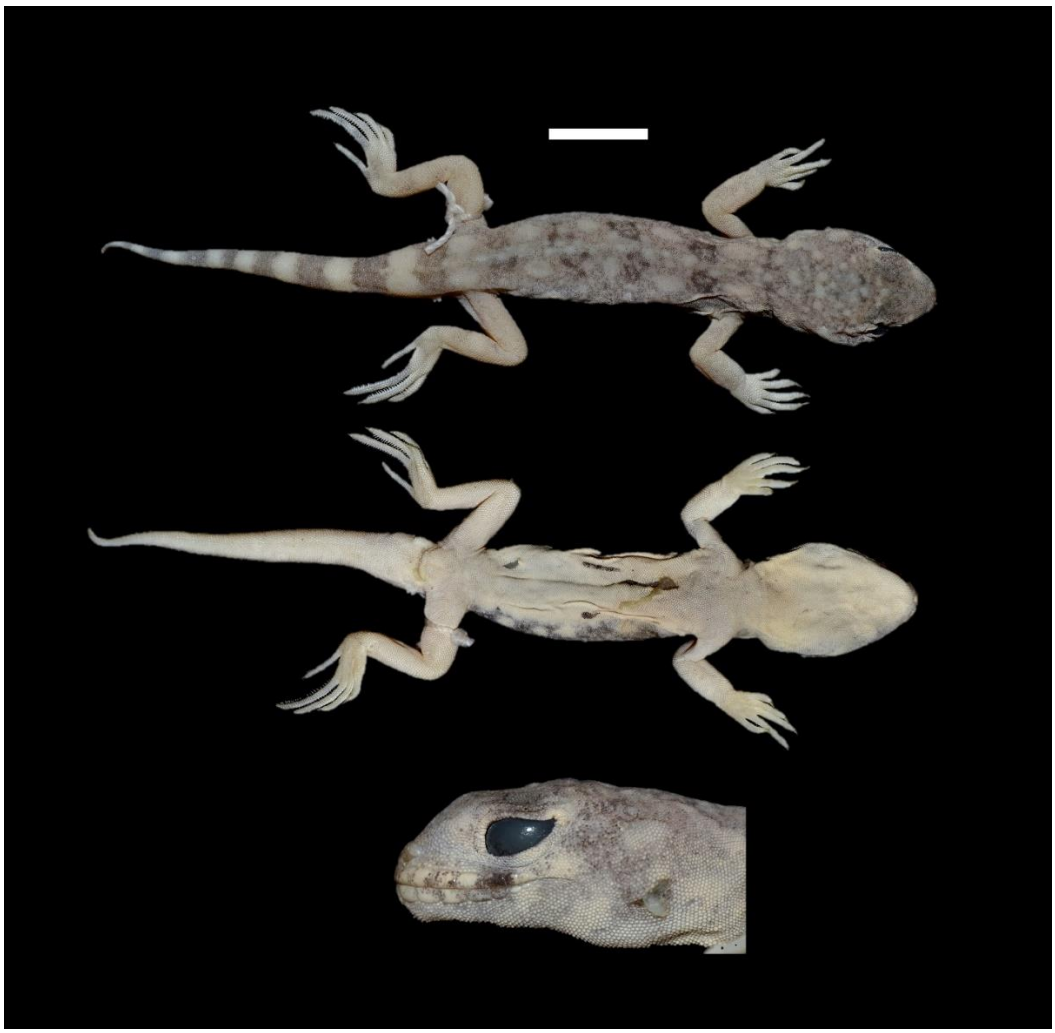

Figure 18: Holotype (NMNW R 11390) of *Ptenopus* SP1 from Grosse Brucht Bay, Namibia. Scale bar = 1 cm relative to full ventral and dorsal views.

Colouration. In life (Fig. 19), dorsal background colour is dull orange with light grey-purple and cream spots; four pairs of large, cream/white, ovoid markings on the back interspaced by diffuse, mottled, dark brown-purple patches; these markings coalesce into alternating cream and light purple/brownish bars on the tail (9 dark bars, the last two very faint). Laterally the colours and markings fade, with immaculate white ventrum. Laterally, the body has five white circular markings, dorsally outlined with light brown/purple. Yellow gular patch extensive, with additional yellow spots on the snout, laterally on the head, body and neck, and on anterior surfaces of the back legs.

In preservative (Fig. 18), the lighter colours faded to off-white or beige and all the darker colours appear various shades of dark brown or grey, while the immaculate white areas appear more cream. The yellow has faded completely.



Figure 19: *Ptenopus* SP1 in life (NMNW R 11390), from Grosse Brucht Bay, Namibia (-26.7208, 15.1026, 45 m). Photo by Francois S. Becker.

Additional material variation. See Table 16 for allotype measurements; see Appendix 4: Table S 3. Internasal scale typically small and asymmetrical; rostral typically narrow and tall; toe fringes relatively extensive. Dorsal colouration highly variable, matching the wide variation of substrate colours and surface textures across its range. Northern populations (including the allotype) have a similar colour pattern to the holotype, whereas the southern populations have a more finely speckled or dotted appearance somewhat similar to *P. garrulus*. The northern populations occur more often on deflation plains with pebbles or course sand on the surface, whereas the southern populations tend to occur in fine sandy substrates – which may account for the colour pattern difference (this appears to be the pattern across the genus). Observed colour variations include overall cream, greyish, ochre, or even brick red background colouration with speckling colour variations including brown, white, olive, purple, orange, and black. Ventrally, animals are immaculate white, although some southern populations possess black or dark grey speckling on the throat and lateral edges of the body, occasionally intruding onto the belly. Males have bright yellow gular patches which often extend onto the lateral head and body, and yellow on anterior surfaces of the hind leg.

Advertisement call. The advertisement call (Fig. 20) consists of 5 notes (range 4 – 6), uttered slowly at a rate of 2.81 s^{-1} (range 2.64 – 3.15). Note duration is short (29 ms [range 24 – 34]) and highly consistent; note 1 duration deviance 9.2% (range -4 – 16). Inter-note intervals 313 ms (range

274 – 341), with a notably longer final interval; inter-note interval range high, 52.2% (range 36 – 66). Median call density is low, 0.09 (range 0.08 – 0.11); call duration 1.4 s (range 1.1 – 1.8). The basal frequency is ~415 Hz (range 370 – 516, but very soft and may be inestimable, with harmonic bands louder towards the (upper) dominant frequency at 4.4 kHz (range 4.1 – 5); usually no notable lower peak frequency peak is evident. Frequency appears to remain more or less constant throughout the note, and (human) perceived pitch is notably lower in northern than southern populations, and also coastal compared to inland ones. Bandwidth (90%) is difficult to estimate consistently: approximately 0.5 – 7.3 kHz.

In coastal populations this species does not call often, but inland populations are usually more vocal, consistent with higher incidence of foggy, cold, or windy weather at the coast in which calling activity is reduced. Call period (5 – 20 s, mean 14 s) is short compared to most other species during peak chorus activity. It mainly calls from about 30 minutes before sunset to dusk, with occasional calling later at night. This species also calls sporadically throughout the day, particularly under foggy or cloudy conditions. This behaviour may reflect the reference made by A. Smith (1849) in his description of the calling behaviour of *P. garrulus*, after his travels through Namaqualand.

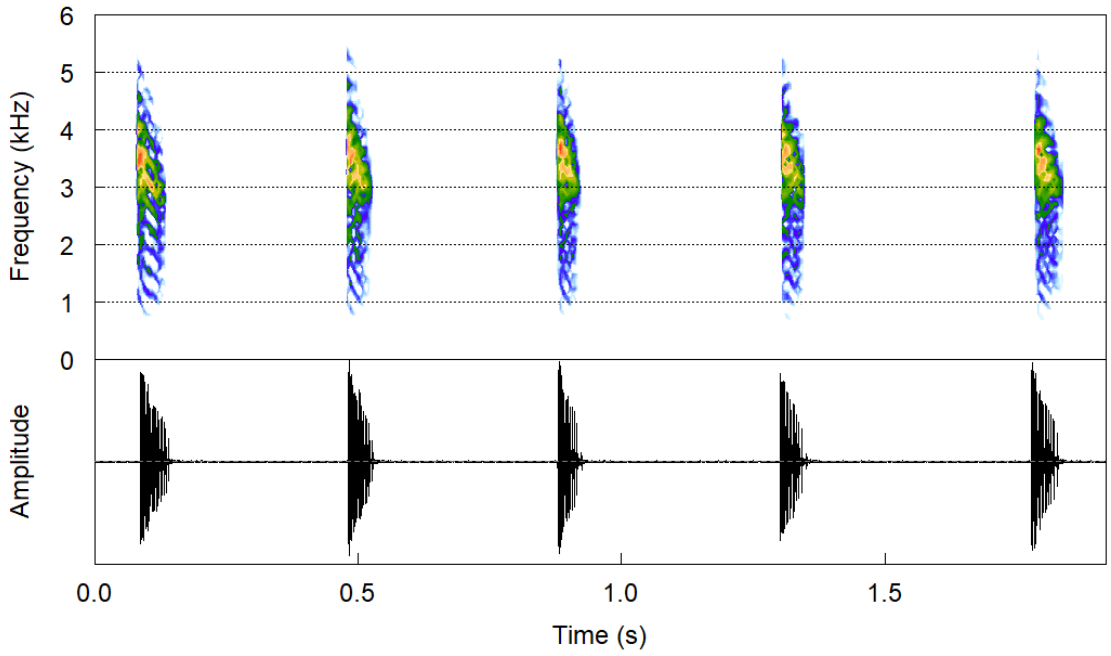

Figure 20: Sonogram (top) and oscillogram (bottom) of a typical advertisement call of *Ptenopus* SP1 (FB1114) from inland of Lüderitz, Namibia (-26.693, 15.406, 321 m). In the sonogram, warmer colours (to red) are higher sound energy, cooler colours (to blue) are lower sound energy.

Habitat and distribution. (Fig. 7). This species occurs on coastal dunes, sandy plains, and on deflation plains in the southern Namib Desert, and/or the Namaqualand-Richtersveld steppe ecoregion (see Dinerstein et al. 2017). It occurs from the edge of the intertidal zone, inland to about 50 – 80 km from the coast. The habitat falls within the coastal fog zone and receives rain predominantly in the austral winter.

Natural history. This species often wanders on the surface throughout the night, far from its burrow, and may be active despite cold, foggy weather, similar to *P. carpi* and *P.* SP4 The burrows are relatively complex with multiple side-tunnels, and a few egg clutches, usually two eggs but occasionally one, have been found inside burrows occupied by a female. This species occasionally closes the burrow entrance from the inside before daybreak, but may also be active diurnally, especially after or during foggy weather. Hatchlings have been observed digging their own burrows, and it is therefore expected that they do not remain in the parent burrows for any length of time. Males and females have not been observed sharing a burrow. This species has been observed feeding on spiders and termites, and it likely eats other arthropods.

Ptenopus SP2

Figs. 21–23

Synonym. *Ptenopus garrulus maculatus* (in part) – Brain, 1962a: 3.

Note. Specimens of this species (referred to in Chapter 3 as clade *P. g. m.* Central) were not included in the earliest mentions of the name *P. g. maculatus* (e.g., Fitzsimons 1935), but were included in several later works, including notably those of Haacke (1964; 1969; 1975). Haacke (1969) described the calls of both *P. maculatus s. s.* and *P.* SP2 from the area surrounding Gobabeb, under the subspecies name *P. g. maculatus*. Haacke and previous authors never managed to match the two distinct calls with particular specimens in areas where they are sympatric, on the gravel plains of the central Namib Desert. In these areas, these two species can be readily distinguished by the internasal scale in *P.* SP2 being large and in broad contact with the rostral, separating the nasal scales clearly, whereas *P. maculatus* has one or more small and rounded granules, barely separating the nasal scales. This morphological distinction is less clear in allopatric populations.

Holotype. NMNW R 11394, adult male, collected from the interdunes plains south of the Kuiseb River from Gobabeb Research Station, Namibia (-23.57053, 15.03618, 415 m), by Francois Becker on 6 September 2022.

Allotype. NMNW R 11395, adult female, with the same collection details as the holotype.

Additional material examined. See Appendix 4: Table S 3 and Appendix 5, Table S 5 for specimens and photographed, non-vouchered material examined (n = 33).

Diagnosis. A moderately small *Ptenopus* (SVL max 54.9 mm, mean 48.6 mm, n = 16) with a short tail (TL 62% [range 48 – 73] of SVL, n = 8) and a lean appearance. This species is easily distinguished from *P. kochi, P. carpi* and *P.* SP4, and *P. garrulus*, but is morphologically similar to *P. maculatus*, *P.* SP3 and *P.* SP1 (previously included in the subspecies *P. g. maculatus*), although it is notably smaller than *P. maculatus.* It differs from the former (more different) species group by the following characters: toes being intermediately fringed laterally (vs. weakly fringed in *P. carpi* and *P.* SP4 and extensively fringed in *P. kochi*, and *P. garrulus*), with fringe length being at least half the breadth of the toe between fringes (vs. generally less than half in *P. carpi* and *P.* SP4, generally equal to in *P. kochi* and *P. garrulus*), although fringing can be more extensive in specimens found in looser sand, such as close to Walvis Bay or Far East Dunes; having white pigmented ventral scales on entire ventral surface (similar to *P. carpi* and *P.* SP4, vs. pink, unpigmented patches on the tail and limbs in *P. kochi*, and pink unpigmented scales on the [hand/foot] palms/soles of *P. garrulus*); having MBSR 139 (108 – 179) (less than, but overlapping with *P. garrulus,* more than, but overlapping with *P. carpi* and *P.* SP4, vs. 187 – 222 in *P. kochi*), with finer lepidosis presenting in specimens found in looser sand; by dorsal colour pattern, characterised by paired, light ovoid markings interspaced by dark mottled patches (vs. spotted pattern in *P. kochi* and most *P. garrulus* populations and banded pattern in *P. carpi* and *P.* SP4). It is further distinct from *P. carpi* and *P.* SP4 by the nasals being more swollen and the nostrils partially covered by internal projections of the upper labials; from *P. kochi* by having fingers laterally fringed with pointed triangular scales (vs. elongated pointed scales in *P. kochi*).

From the latter group of species in the *P. garrulus* complex, *P.* SP2 is distinguished by the internasal scale and rostral being larger and/or broader, with IN/INSBB 4.49 (3.7 – 5.3) (overlapping with *P. maculatus,* vs. 6.5 – 11.0 for *P.* SP3 and 5.2 – 10.2 for *P.* SP1), RB/RH = 1.29 (1.2 – 1.4) (overlapping substantially with *P. maculatus*, vs. 1 – 1.4 in *P.* SP3 and 0.9 – 1.1 in *P.* SP1), and INSH/NB 1.57 (0.99 – 1.79, overlapping substantially with other species, vs. 0.55 – 1.03 in *P.* SP1); by lower MBSR = 139 (108 – 179), but similar to *P. maculatus*. It is further distinct from *P. maculatus* by being smaller and having higher IOS count = 46 (37 – 51), vs. 32 – 40 in *P. maculatus*.

Its advertisement call is distinguished from that of all other species in the genus mainly by a combination of the following characters (see Fig. 9): 5 notes (range 3 – 7) (similar to other *P. garrulus* species vs. > 7 notes in *P. kochi, P. carpi,* and *P.* SP4) with regular duration, uttered in highly regular succession with inter-note interval range 13% (range 10 – 20) – the lowest of all species; note rate (2.81 s^{-1} [range 1.57 – 3.68]) is much lower than *P. kochi* (5.98 – 10.33 s^{-1}), *P. garrulus* (3.76 – 7.37 s^{-1}) and *P. maculatus* (2.58 – 7.17 s^{-1}); median call density (0.13 [range 0.09 – 0.17]) is notably higher than *P.* SP1 (0.08 – 0.11), *P. carpi* and *P.* SP4 (0.05 – 0.11) and notably lower than *P.*

kochi (0.20 – 0.34), *P. maculatus* (0.18 – 2.98) and *P.* SP3 (0.18 – 0.48). Audible (for humans) pitch is sometimes lower than sympatric *P. maculatus,* although the upper dominant frequency is higher (3.82 kHz [range 3.5 – 4.1], vs. *P. maculatus* = 3.38 kHz [range3.0 – 3.8]).

Holotype description. (Fig. 21). Adult male, SVL 48.75 mm with original tail 35.79 mm (73.42% SVL). Body and head covered with minute hexagonal to round scales of a similar size, dorsally and ventrally, with scales on limbs notably larger. Small mid-ventral incision for removal of liver sample. Body and head slender, MBSR 129, IOS 53, HL 14.62 mm, HW 10.99 mm, HH 5.57 mm, EED 5.81 mm. Upper and lower labials 8, six granules bordering the mental. Clear but not prominent superciliary ridges tapering above mid-eye level; a single row of elongated scales around the anterior and upper margins of the eye with curved tips posteriorly, the scales being smaller and rounder along the posterior and dorsal edges; nasal scales swollen, the prenasals separated by one large, hexagonal internasal scale in broad contact with the rostral, with IN/INSBB 4.69, INSH/NB 1.09; nostrils partially covered by projection from the upper nasal. Rostral broad, with RB/RH 1.44, MB/RB 0.81. Eyes intermediate, EYE 3.15 mm, pupil vertical. Ear opening is oblique (~45°) and narrow, with small, slightly projecting scales at the anterior margin. Toes elongate, flattened, with elongate fringed scales, small, pointed, triangular fringes on the fingers; strong nails on fingers and toes, being larger and thicker on the fingers.



Figure 21: Holotype (NMNW R 11394) of *Ptenopus* SP2 from Gobabeb, Namibia. Scale bar = 1 cm relative to full ventral and dorsal views.

Colouration. In life (Fig. 22) dorsal background colour is ochre with deep orange, cream, and dark brown spots, the darker spots aggregated in clusters; three pairs of larger, cream/light yellow, ovoid markings from the neck to the back, with one unpaired marking on the left lower back and another asymmetrical pair onto the tail base; light markings interspaced by diffuse, asymmetrical, mottled or spotted, dark brown patches; the tail has 11 dark bands, unevenly spaced and somewhat diffuse, with the last being very faint; the dorsal colours and markings fade to grey on the lateral edges with clear alternating light-and-dark markings; immaculate white ventrum. Yellow gular patch with faded edges.

In preservative (Fig. 21), the lighter colours have faded to off-white or beige, and all the darker colours appear various shades of dark brown or grey with some orange evident. The yellow has faded completely.



Figure 22: *Ptenopus* SP2 in life (NMNW R 11394), from south of the Kuiseb River near Gobabeb Research Station, Namibia (-23.5732, 15.0368, 415 m). Photo by Francois S. Becker.

Additional material variation. See Table 16 for allotype measurements; see Appendix 4: Table S 3. Internasal scale typically large and symmetrical; rostral typically broad; toe fringes relatively extensive, notably more so in areas with looser sand. As with other species, the colour of the animals usually matches that of the local substrate, although the patterning is similar to the holotype. Background colour variations include ochre to brick red. Ventrally animals are immaculate white. Males have faded to bright yellow gular patches (typically less extensive than most other species), occasionally with slight yellow on hidden dorsal surfaces of the hind leg.

Advertisement call. The advertisement call (Fig. 23) consists of 4.6 notes (range 3 – 7), uttered slowly at a rate of 2.44 s^{-1} (range 1.57 – 3.68). Note duration is long (48 ms [range 31 – 57]) and regular (note 1 duration deviance 16% [range 3 – 28]), with long, highly regular intervals (inter-note interval range 13% [range 10 – 20]) inter-note intervals of 385 ms (range 237 – 587). Median call density is low (0.129 [range 0.09 – 0.17]) and call duration is 1.49 s (range 1.1 – 2). The basal frequency is ~ 383 Hz (range 308 – 474), but very soft and may be inestimable, with harmonic bands louder towards the (upper) dominant frequency at 3.8 kHz (range 3.5 – 4.1); no clear lower peak frequency is evident. Frequency appears to modulate up by ~0.5 kHz in the course of the note, but (human) perceived pitch is usually monotonous throughout the notes and call. Bandwidth (90%) is difficult to estimate consistently: approximately 1 – 4.7 kHz.

The call strongly resembles the sound of bouncing marbles and on a warm evening on the interdunes thousands can be heard calling in chorus. Call period (mean 133 s) varies greatly, but can be as brief as 16 seconds during peak chorus activity. This species tends to occur (and call) at higher

densities than the sympatric congeners *P. maculatus* and *P. kochi*. It calls from shortly before sunset and calling activity may continue throughout the night after moonrise and before moonset, particularly on warm evenings. This species occassionally calls during the day, especially during foggy or cloudy conditions, but generally not during rainy conditions.

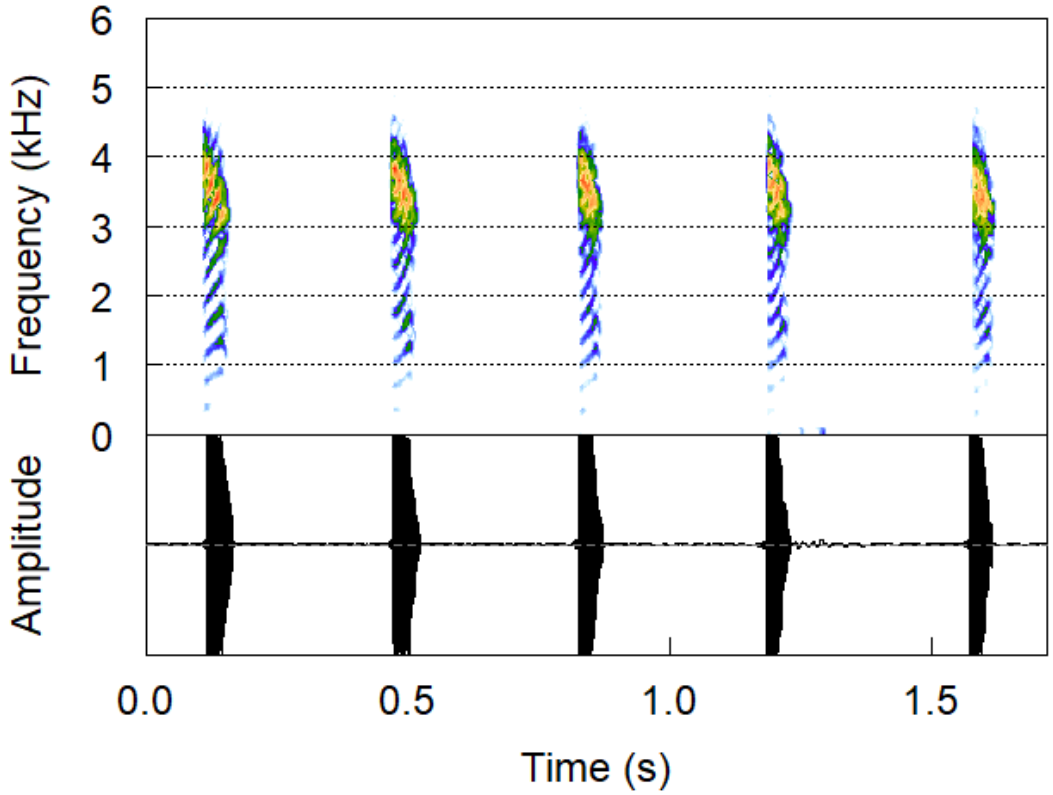

Figure 23: Sonogram (top) and oscillogram (bottom) of a typical advertisement call of *Ptenopus* SP2 (FB878) from near Gobabeb Research Station, south of the Kuiseb River, Namibia (-23.5732, 15.0398, 415 m). In the sonogram, warmer colours (to red) are higher sound energy, cooler colours (to blue) are lower sound energy.

Habitat and distribution. This species occurs on sandy soils including interdune plains within, and sandy plains around the northern and eastern edges of the Namib Sand Sea Namibia (Fig. 7). It generally does not occur on the gravel plains north of the Kuiseb River except in a few sandier patches east of 15° longitude. This population is genetically divergent from other nearby populations in the Sand Sea (Chapter 2). It does not appear to occur along the south-eastern corner of the Namib Sand Sea, where slightly harder Arenosols are occupied by *P.* SP3 sp. nov, or along the southern or south-western edges of the sand sea where the same soils are occupied by *P.* SP1

It has not been found in sympatry with *P.* SP3, but overlap is likely in the southern portion of its range. It occurs sympatrically with *P. maculatus* on the sandier patches of the gravel plains north of the Kuiseb, but in allopatry further east along the northern margins of the Kuiseb Canyon, where

the plains are sandiest. It frequently occurs in sympatry and syntopy with *P. kochi* in the Namib Sand Sea, near the edges of the interdunes where the two species' habitats overlap, but occurs in allopatry on the central parts of the interdunes (while *P. kochi* prefers the dune plinth).

Natural history. Two eggs (dried out) have been recovered from an abandoned burrow, so clutch size can be up to two. The burrows are generally less complex than the sympatric *P. kochi* and *P. maculatus*, and appear to be inhabited solitarily. Very small juveniles have been observed in burrows of their own. While multiple natural predators for *Ptenopus* spp. have been observed and hypothesised (Haacke 1975), one observation of a *P.* SP2 being varied off by a large solifugid (le Roux pers. com.) certainly constitutes a novel observation.

Ptenopus SP3

Figs. 24–26

Synonym. *Ptenopus garrulus maculatus* (in part) – FitzSimons, 1935: 525.

Note. Specimens of this species (referred to in Chapter 3 as clade *P. g. m.* East) were first included under the subspecific name *P. g. maculatus* by FitzSimons (1935) and subsequently by several authors, including the notable generic revision by Haacke (1975). Its advertisement call has not been explicitly described. All *Ptenopus* specimens that occur on compact substrates in the south of Namibia, inland, and some from the Great Karoo (Northern Cape) are likely to be assignable to this species.

Holotype. NMNW R 11389, adult male, collected from farm Goris near Giants' Playground, Namibia (-26.454, 18.310, 1106 m), by Francois Becker and Bertha Buiswalelo on 27 September 2022.

Allotype. NMNW R 10839, adult female, collected from Koës, Namibia (-25.937, 19.085, 1004 m), by Francois Becker, Hileni Shivolo and Sisamu Baepi on 6 October 2020.

Additional material examined. See Appendix 4: Table S 3 and Appendix 5, Table S 5 for specimens and photographed, non-vouchered material examined (n = 26).

Diagnosis. A medium-sized *Ptenopus* (SVL max 58 mm, mean 51.3 mm, n = 10) with a short tail (TL 65% [range 60 – 72] of SVL, n = 7) and a stout appearance. It is distinguished from all congeners by a combination of the following characters: toes being intermediately fringed laterally (similar to other species vs. weakly fringed in *P. carpi* and *P.* SP4 and extensively fringed in *P. kochi* and *P. garrulus*), with fringe length being at least half the breadth of the toe between fringes (vs. generally less than half in *P. carpi* and *P.* SP4, and generally equal to in *P. kochi* and *P. garrulus*); having white pigmented ventral scales on entire ventral surface except some black or grey speckling laterally and on the (hand/foot) palms/soles (vs. pink, unpigmented patches on the tail and limbs in

P. kochi, and pink unpigmented scales on the [hand/foot] palms/soles of *P. garrulus,* and entirely white in other species, although dark specking on throat and body may occur in *P.* SP1,); having MBSR 144 – 180, mean 158 (n = 13) (similar to *P. g. garrulus* and *P.* SP1, vs. 187 – 222 in *P. kochi*, and generally < 150 in other species); by having on the dorsum large, light, paired ovoid markings interspaced or dominated by dense dark brown mottled patches (vs. a spotted or speckled pattern in *P. kochi* and *P. garrulus*, banded pattern in *P. carpi* and *P.* SP4, but similar to the other species). It is further distinct from *P. carpi* and *P.* SP4 by the nasals being more swollen and the nostrils partially covered by internal projections of the upper labials; from *P. kochi* by having fingers laterally fringed with pointed triangular scales (vs. elongated pointed scales in *P. kochi*); from *P.* SP2 by a smaller internasal scale, with IN/INSBB 6.2 – 11 (vs. < 6 in *P.* SP2).

Its advertisement call is distinguished from that of all other *Ptenopus* species mainly by a combination of the following characters (see Fig. 9): 5 notes (range 4 – 5) (similar to other species, vs. > 7 notes in *P. kochi, P. carpi,* and *P.* SP4); the longest note duration (92 ms [range 61 – 154]) of all species (vs. < 72 in *P. maculatus* and < 60 ms in other species); higher median call density (0.29 [range 0.18 – 0.48]) than other species except for *P. maculatus* and *P.* kochi; note 1 duration deviance 30% (range 10 – 47) (vs. 106 – 174% in *P. maculatus* and usually < 30% for other species).

Holotype description. (Fig. 24). Adult male, SVL 51.41 mm with original tail 30.61 mm (59.54% SVL). Body and head covered with minute hexagonal to round scales of a similar size, dorsally and ventrally, with scales on limbs notably larger. Small mid-ventral incision for removal of liver sample. Body slender, MBSR 166, IOS 48, HL 15.97 mm, HW 11.15 mm, HH 6.85 mm, EED 5.93 mm. Upper labials 6 enlarged, with 4 – 5 small granules up to the angle of the jaw, lower labials 8, the posterior scale elongate and thin, and six granules bordering the mental. Clear but not prominent superciliary ridges tapering from above mid-eye level. Around the eye is a single row of elongated scales around the anterior, dorsal, and posterior margins, but more rounded and smaller along the ventral margin. Nasal scales slightly swollen, the prenasals barely separated by one small, rounded internasal scale in meagre contact with the rostral, with IN/INSBB 8.71, INSH/NB 1.03. Nostrils partially covered by projection from the upper nasal. Rostral broad, with RB/RH 1.42, MB/RB 0.75. Pupil vertical, EYE 3.46 mm. Ear opening is oblique (~45° ref) and narrow with small projecting scales at the anterior margin. The neck region behind the cheeks is slightly swollen with internal calcium deposits. Arms, legs, and tail stout in appearance. Toes elongate, flattened, with moderately elongate fringed scales, small, pointed, triangular fringes on the fingers; strong nails on fingers and toes, being larger and thicker on the fingers.

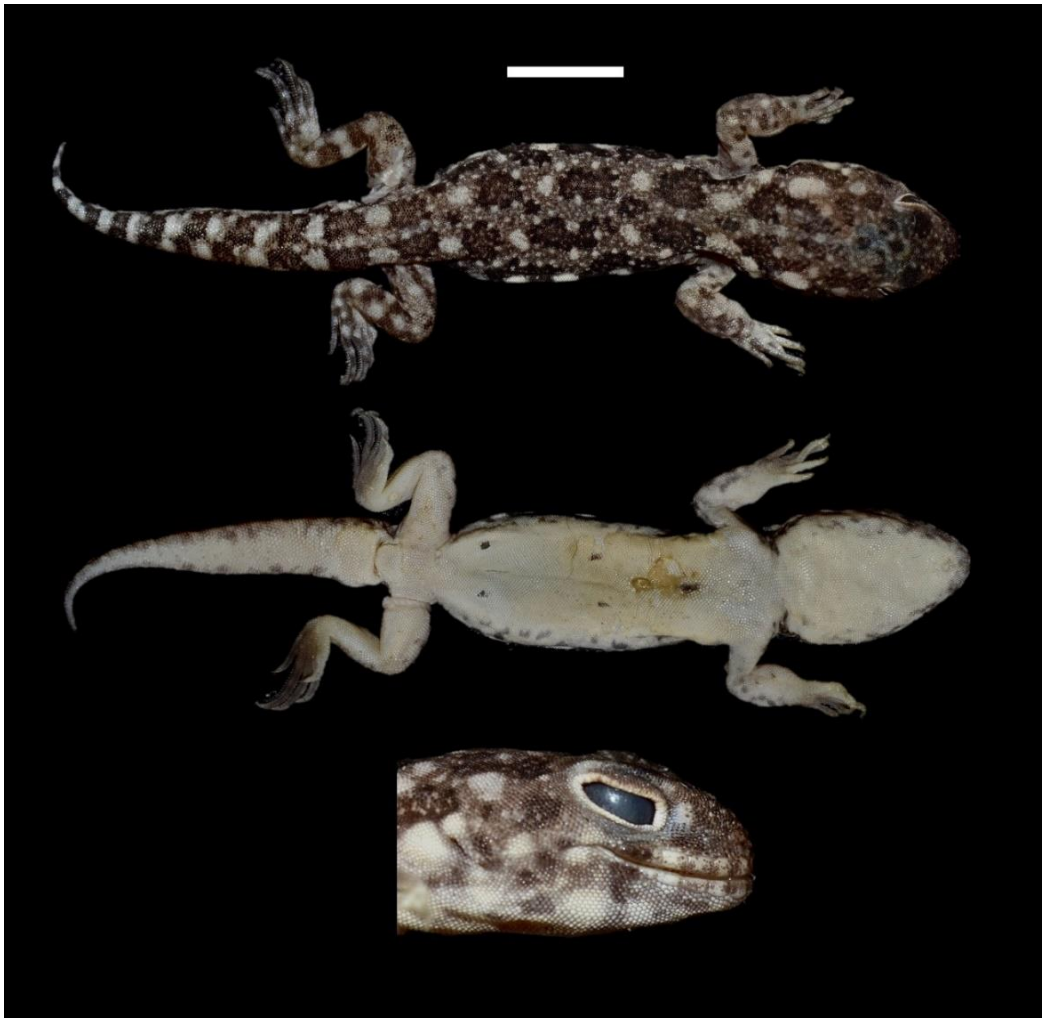

Figure 24: Holotype (NMNW R 11389) of *Ptenopus* SP3 from Giants' Playground, Namibia. Scale bar = 1 cm relative to full ventral and dorsal views.

Colouration. In life (Fig. 25) dorsal background colour is light brown with orange, cream, and beige spots, with five intermediate paired, circular, markings on the neck and back interspaced by larger dark brown, irregular markings and some smaller cream dots and dark blotches that are more-or-less symmetrically scattered across the dorsal surface; the paired cream markings merge into bands on the tail (somewhat asymmetrical in the mid-tail) interspaced by 10 – 11 dark bars, which are barely noticeable on the nearly white tail tip; prominent dark brown to black dorsolateral markings; ventrum is immaculate white except for some dark grey mottling laterally, on ventral leg surfaces, and substantial dark mottling on (hand/foot) palms/soles. Extensive, bright yellow gular patch with faded anterior edges and a clear but speckled posterior edge, with a large white area on the throat; the bright yellow does not extend onto the lateral surfaces of the head, body, or onto the limbs.

In preservative (Fig. 24), the lighter colours have faded to off-white or beige, the darker colours remain various shades of dark brown or grey, and the orange and yellow colours have faded completely.



Figure 25: *Ptenopus* SP3 (NMNW R 11389) in life, from farm Goris near Giant's Playground (-26.4538, 18.3097, 1106 m), Namibia. Photo by Francois S. Becker.

Additional material variation. See Table 16 for allotype measurements; see Appendix 4: Table S 3. Scalation varies relatively little among specimens, although the colour patterns vary substantially, with background colouration variations including brown, light orange, beige, or red-brown; some amount of orange speckling is usually present. Ventrally, animals are immaculate white with grey or brownish speckling clearly visible on (hand/foot) palms/soles. Males have bright yellow gular patches, while some male specimens also have yellow on the anterior or dorsal surfaces of the hind leg.

Advertisement call. The advertisement call (Fig. 26) consists of 4.6 notes (range 4 – 5), uttered slowly at a rate of 2.45 s^{-1} (range 1.99 – 2.96). Note duration is the longest of all species (92 ms [range 61 – 154]), the first usually notably longer than those following, with note 1 duration deviance 30% [range 10 – 47]. Inter-note intervals are 316 ms (range 269 – 368) and quite regular, inter-note interval range 23.3% (range 12 – 42). Median call density is high, 0.29 (range 0.18 – 0.48). Call duration is 1.57 s (range 1.3 – 1.9). The basal frequency is ~399 Hz (range 366 – 474), but very soft may be inestimable, with harmonic bands louder towards the dominant frequency at 3.506 kHz (range 3.3 – 3.8); sometimes a very slight lower frequency peak is evident at around or just below half the dominant frequency (roughly 1.2 – 1.9 kHz, cannot always be reliably estimated). Frequency appears to modulate down by ~0.4 kHz during the course of the note, and (human) perceived pitch

also seems to modulate within each note, slightly reminiscent of dripping water. Bandwidth (90%) is difficult to estimate consistently: approximately 0.5 – 6.9 kHz.

Calling activity for this species commences ~ 30 min before sunset and may continue late into the night if the moon is up. Call period (mean 84 s) varies considerably, but can be as low as 18 seconds during peak chorus activity.

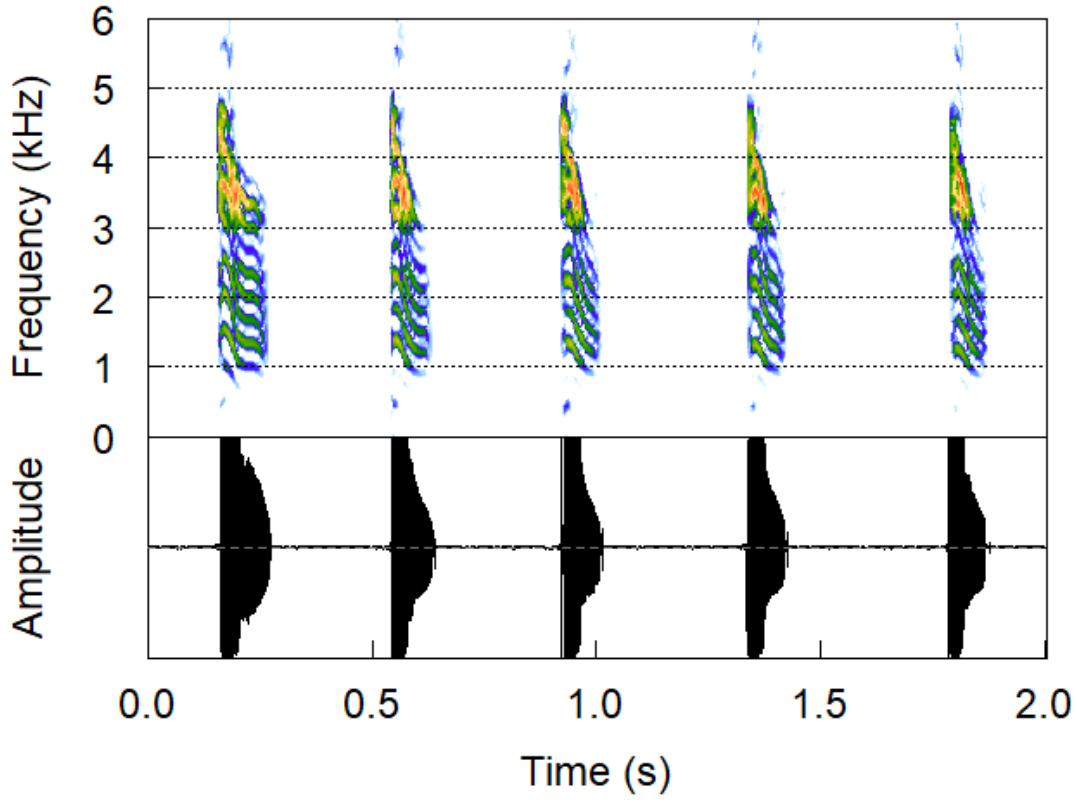

Figure 26: Sonogram (top) and oscillogram (bottom) of a typical advertisement call of *Ptenopus* SP3 (FB902) from near Keetmanshoop, Namibia. In the sonogram, warmer colours (to red) are higher sound energy, cooler colours (to blue) are lower sound energy.

Habitat and distribution. This species occurs on consolidated soils such as gravel, alluvial silt, or compacted sandy soils throughout southern Namibia and may occur widely in the Northern Cape, South Africa (Fig. 7). It probably does not occur on the rocky formations in central southern Namibia, but occurs west and east of it. The northeastern edge of its range is the western edge of the Kalahari Sand Sea, as it does not occur on loose sand. There is only one confirmed record from South Africa near Onseepkans, Northern Cape, but similar soils occur more broadly throughout large parts of the Northern Cape Province.

It has not been recorded in sympatry with any other species, but occurs parapatrically to *P. garrulus* near Koës, Namibia (with *P. garrulus* occurring in the Kalahari Sand Sea), and in the Northern Cape, South Africa (where patches of Kalahari sand occur on more consolidated soils). It

has been recorded ~40 km from the nearest confirmed *P.* SP1 record in the west, and no closer than ~60 km from the nearest confirmed *P.* SP2 record in the northwest of its range.

Natural history. Calling (and therefore breeding) activity appears to be seasonal, probably peaking in the austral spring. This species is seldom found on the surface, except early in the evening during peak chorus activity. Burrow entrances are nearly always sealed after calling ceases or during the day; the entrance is sealed from the inside, and it is so neatly disguised as to be almost entirely indistinguishable from the surrounding soil surface. The burrow is then re-opened before calling commences. It has been observed feeding on harvester termites (*Hodotermes*) even in small groups, and these have been observed occurring at each site where the geckos were collected. Aggregations of this species are sometimes observed on the tar road late at night (not observed in other species), although the trigger for this behaviour is not yet understood.

Ptenopus SP4

Figs. 27–29

Synonym. *Ptenopus carpi* (in part) – Haacke, 1975: 230.

Note. This species (referred to in Chapter 3 as clade *P. carpi* B) is closely related to *P. carpi* but occurs predominantly north of the Swakop River, while *P. carpi* only occurs to the south. A single call from one individual in captivity was previously described by Gramentz (2008) as *P. carpi*, although the call and its variation is herein re-assessed and fully described in a standardised taxonomic framework.

Holotype. NMNW R 13c, adult male, collected from Möwe Bay, Skeleton Coast National Park, Namibia (-19.372, 12.710, 30 m), by C. G. Coetzee, M. L. Penrith, and B. F. Kensley, on 1 June 1969.

Allotype. NMNW R 13h, adult female, same collection details as the holotype.

Additional material examined. See Appendix 4: Table S 3 and Appendix 5, Table S 5 for specimens and photographed, non-vouchered material examined (n = 35).

Diagnosis. A moderately large *Ptenopus* (SVL max 57.1 mm, mean 52.7 mm, n = 18) with a moderate tail (TL 70% [range 62 – 80] of SVL, n = 12), an overall lean appearance and comparatively slender limbs. It is morphologically indistinguishable from *P. carpi* except by sexual dichromatism, with females no yellow gular patch as is typical for the genus (vs. female gular patch in *P. carpi*); by silver to brown iris colouration (vs. red or ochre in *P. carpi*). It is distinguished from all other congeners except for *P. carpi* by a combination of the following characters: banded dorsal colour pattern on the body and tail (vs. spotted or speckled pattern in *P. garrulus* and *P. kochi,* and large, paired, ovoid light markings interspaced by darker mottled patches in other species); weakly fringed

toes, with fringe length being generally less than half the breadth of the toe between fringes (vs. more than half for other species); nostrils not covered internally by a projection from the upper nasal scale (vs. is covered partially or completely in other species).

Its advertisement call is distinguished from that of all other *Ptenopus* species by a combination of the following characters (see Fig. 9): 10 notes (range 8 – 13) (similar to *P. carpi*, vs. 10 – 19 notes in *P. kochi* and < 8 notes in all other species); inter-note intervals normally increasing notably as the call progresses (vs. shortest in the middle for *P. carpi*, a notably longer last interval in *P.* SP1, and relatively consistent for all other species), being usually highly variable (inter-note interval range 102% [range 26 –163], vs. 62 – 105% in *P. carpi*, 36 – 66% in *P.* SP1, and < 30% in all other species); note duration highly regular (distinct from *P. maculatus* and *P.* SP3). It is further distinct from *P. carpi* by a higher upper dominant frequency (4.3 kHz [range 3.8 – 4.8] vs. 3.5 kHz [range 3.3 – 3.8] in *P. carpi*).

Holotype description. (Fig. 27). Adult male, SVL 52.0 mm with original tail 37.1 mm (71.25% SVL). Body and head covered with minute hexagonal to round scales of a similar size, dorsally and ventrally, with scales on limbs notably larger. Body slender, MBSR 131, IOS 40, HL 15.45 mm, HW 11.58 mm, HH 7.62 mm, EED 5.9 mm. Upper labials 9 enlarged, with 1 additional small scale at the angle of the jaw; lower labials 9 enlarged and one small scale posteriorly; five granules bordering the mental. Clear superciliary ridges tapering from above mid-eye level. Around the eye is a single row of elongated scales around the anterior, dorsal, and posterior margins, but more rounded and smaller along the ventral margin. Nasal scales very slightly swollen, the prenasals clearly separated by one large, hexagonal internasal scale in broad contact with the broad rostral. Eyes large, EYE 4.22 mm. Ear opening oblique (~45°), very narrow, set on the far posterior corner of the head, with small projecting scales at the anterior margin. The neck region behind the cheeks is very slightly swollen with internal calcium deposits. Arms, legs, and tail lean in appearance, with a slightly bugling stomach. Toes elongate, moderately flattened and weakly fringed; small, pointed fringed scales on finger margins; strong nails on fingers and toes, being larger and thicker on the fingers.



Figure 27: Holotype (NMNW R 13c) of *Ptenopus* SP4 from Möwebay, Skeleton Coast National Park, Namibia, Namibia. Scale bar = 1 cm relative to full ventral and dorsal views.

Colouration. In life, referring to additional material (e.g., Fig. 28) below.

In preserve (holotype), dorsally three broad, brownish bands on the body and seven on the tail, with slight darker band on anterior potions of the head, all interspaced by cream to beige bands speckled with brown; limbs faintly speckled, hind limbs very faintly banded; ventrally uniform beige or cream with dark, discoloured belly.

Additional material variation. See Table 16 for allotype measurements. Scalation varies relatively little among specimens, although the colour patterns vary somewhat. In life (Fig. 28), the dorsal colour pattern consists of 3 – 4 dark brown or purplish bands on a beige, yellow or light orange background, densely speckled with dark brown, purple and/or sometimes green; sometimes the speckling is very dense, giving the appearance that the gecko is not or barely banded; an additional six to eight dark bands occur on the tail. Banding tends to be clearer and colouration brighter in younger or smaller individuals. Males have bright yellow gular patches, and yellow may

extend onto the snout. Females lack the bright yellow gular patch, but may have traces of faded yellow on the lateral margins of the throat.



Figure 28: Adult male *Ptenopus* SP4 (FB373) in life from Henties Bay, Namibia (-22.158, 14.309, 13.6 m), Namibia. Photo by Francois S. Becker.

Advertisement call. The advertisement call (Fig. 29) consists of 10 (8 – 13) notes uttered in slow succession, with a note rate of 2.60 s^{-1} (1.25 – 4.32). Note duration is short (28 ms [range 21 – 40]) and regular, note 1 duration deviance 8.7% (range 0.5 – 16.2). The inter-note intervals are very long (387 ms [range 204 – 661]) and usually irregular, the inter-note interval range being high (102% [range 26 – 163]), with intervals becoming progressively longer as the call progresses. Median call density very low (28.3 [range 20.9 – 40.3]) and call duration is long (3.9 s [range 2.5 – 7.2]). The basal frequency is 405 Hz (range 357 – 516) but very soft and usually inestimable, with harmonic bands louder towards the dominant frequency. The upper dominant frequency is at 4.3 kHz (3.8 – 4.8), and a clear (sometimes louder) lower dominant frequency is evident at around 2.0 kHz (1.4 – 1.9), usually less than half the upper dominant frequency. Frequency appears to remain constant throughout the notes and call, as is the case with the (human) perceived pitch, which is low and monotonous compared to most other species. Bandwidth (90%) is difficult to estimate consistently, but is broad: approximately 0.3 – 6.9 kHz.

The call seems to vary more than other species, except for *P. carpi*, with calls uttered late at night sometimes having only one to three notes. Call period (mean 365 s) varies greatly, but is usually lower than other species, and has not been recorded any faster than 1 min, 44 s during peak

chorus activity. This species does not chorus as notably as most other species. Calls can be heard from after sunset to late at night. Seasonal phenology of calling activity is not known, but calls have been recorded in May and October. Calling activity is more pronounced on warmer evenings, but may continue despite cold, foggy conditions.

As with other species, *P.* SP4 calls from the burrow entrance, although it may also call occasionally while roaming. This could not be observed directly, but a wandering gecko was often spotted in the area where a call was just heard – although wandering geckos are easier to spot than geckos in a burrow.

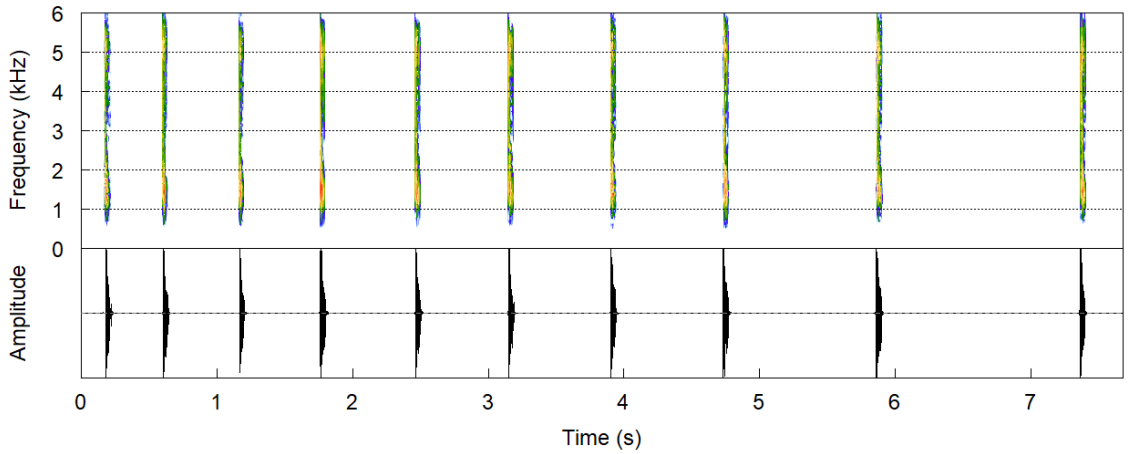

Figure 29: Sonogram (top) and oscillogram (bottom) of a typical advertisement call of *Ptenopus* SP4*s* **sp. nov.** (FB822) from Swakopmund, Namibia (-22.621, 14.537, 15.5 m), Namibia. In the sonogram, warmer colours (to red) are higher sound energy, cooler colours (to blue) are lower sound energy.

Distribution and habitat. This species occurs from the northern banks of the Swakop River, northwards in a narrow strip along the Namibian west coast or Skeleton Coast (Namib Desert), to northwestern Namibia (Fig. 7). Its habitat falls within the coastal fog zone, and it occurs on compact, gravelly substrates. It is not found on soft sand, hence the sand sea just south of the Kunene River, northwestern Namibia, probably marks the northern border of its range.

This species occurs parapatrically with *P. carpi* across the Swakop River. Only one individual was found 2 km south of the Swakop River, but this appears to be a rarity. *P.* SP4 occurs in sympatry with *P. maculatus* in the eastern portions of its range.

Natural history. *Ptenopus* SP4 does not call nearly as often as most congeners, but appears to spend more time wandering on the surface, even far from the burrows (as in *P. carpi* and *P.* SP1). It is active much later into the night than most congeners, often only emerging well after dark, and its activity is not dampened by cold or foggy conditions (as in most other species).

The burrow is usually shallower and simpler than those of other species, but may have two to three side-branches and typically has an escape exit (as in other species). As in *P. carpi,* it utters two different calls: the advertisement call and the single-pulse call, the latter usually uttered late at night after the main calling activity has ceased, and especially during dense fog.

Key to the species of *Ptenopus*

1) Toes weakly fringed; nasals not swollen; nostril open; banded dorsal colour pattern ..... 2)
– Toes strongly fringed laterally; nasals more or less swollen; nostril partly closed by internal projection of upper nasal ..... 3)
2) Iris red or ochre, bright yellow gular patch in both sexes ..... *P. carpi*
– Iris silver or brown, yellow gular patch absent in females ..... *P.* SP4
3) Fingers depressed, fringed with elongated pointed scales; body scales minute (MBSR 187-222), ventrum white, with pink unpigmented patches on tail and limbs ..... *P. kochi*
– Fingers not depressed, edged with pointed triangular scales; body scales usually larger (MBSR 110-194) ..... 4)
4) Extensive fringing on the toes, fringe length roughly equal to breadth of ventral scale attached to fringe, found on more-or-less sandy soils ..... 5)
– Intermediate fringing on the toes, fringe length usually less than breadth of ventral scale attached to fringe, occurs on consolidated soils ..... 7)
5) Ventrum white, with pink unpigmented patches on palms/soles; body scales small (MBSR usually > 160 but can be as low as 140); occurs outside (east of) the Namib Desert ..... *P. garrulus*
– Ventrum and palms/soles immaculate white or occasionally speckled; MBSR usually < 145 (can be as high as 180); occurs in or around the Namib Desert ..... 6)
6) IN/INSBB usually $\leq$ 5.2, INSH/NB usually > 1 RB/RH $\geq$ 1.2, and occurs north of -26° latitude ..... *P.* SP2
IN/INSBB > 5.2, INSH/NB usually < 1, RB/RH < 1.2, occurs south of -26° latitude and < 60 km from the coast ..... *P.* SP1
7) Head scales large, IOS 38 (32 – 40); MBSR 142 (131 – 156); Palms/soles immaculate white; occurs north of -24° latitude ..... *P. maculatus*
– Head scales smaller, IOS 45 (42 – 50), MBSR 166 (160 – 180); palms/soles usually lightly speckled or mottled; occurs south of -24° latitude ..... *P.* SP3

Key to the advertisement calls of *Ptenopus* species

Note: this key is most effective when applied to calls recorded during chorus time; this key is best used in combination with geographic location, as calls are usually more different where species occur adjacent or in sympatry. Also note "exceptions to advertisement call key", below.

1) Full call has > 7 notes ..... 2)
2) Full call has 7 or fewer notes ..... 5)
3) Note rate > 5 s^{-1}, inter-note interval range < 55% (intervals regular),
   median call density > 0.19, notes > 9 ..... *P. kochi*
– Note rate < 5 s^{-1}, inter-note-interval range usually > 55%,
   median call density < 0.12 ..... 4)
4) Upper dominant frequency < 3.8 kHz, inter-note intervals
   decreasing towards the middle of the call, then increasing ..... *P. carpi*
– Upper dominant frequency > 3.8 kHz, inter-note intervals
   Increasing towards the end of the call ..... *P.* SP4
5) Note 1 deviance > 100%, median call density > 0.17 ..... *P. maculatus*
– Note 1 deviance less than 50% ..... 6)
6) Note repetition rate $\geq$ 3.7 s^{-1}, note duration < 32 ms ..... *P. garrulus*
– Note repetition rate < 3.7 s^{-1} ..... 7)
7) Median call density $\geq$ 0.18, note duration > 60 ms, first note
   usually notably longer ..... *P.* SP3
– Median call density < 0.18, note duration < 60 ms ..... 8)
8) Last inter-note interval notably longer than others,
   inter-note interval range > 30% ..... *P.* SP1
– Inter-note intervals range < 30% ..... *P.* SP2

Exceptions to advertisement call key: Number of notes in *P. carpi* can be lower than indicated in the key, sometimes uttering a single note (this type of call appears to have a different function than the advertisement call). Inter-note interval range can be lower (more regular note spacing) than indicated, in some *P.* SP4 individuals around Hoanib River, north-western Namibia. In *P. maculatus* populations in the Hoanib River, north-western Namibia, the note 1 duration deviance is unusually low (~30%). Note 1 duration deviance in *P. kochi* can also be higher than indicated here, in rare individuals north of the Kuiseb River at Gobabeb Research Station (central Namib Desert, Namibia), where *P. kochi* occur sympatrically with *P. maculatus*. Two of these individuals captured were morphologically intermediate between *P. kochi* and *P. maculatus*, and may be hybrids.

Discussion

Thanks to new insights from phylogenetics (Chapter 2) and a thorough reinvestigation of advertisement call variation (Chapter 3), the new taxonomic model for the genus *Ptenopus* now contains eight species. The genus is highly conserved morphologically, with relatively few diagnostic traits to distinguish most species. However, clear differences in advertisement calls, preferred soil texture, and habitat can be observed among the eight species. Very little is known about the ecology and life-history traits of most species, although some similarities occur throughout the genus.

While most morphological and call characteristics indicate probable conservatism, there is also some evidence for ecological convergence in some traits. For example, *P. maculatus* is sister to *P. kochi*, despite being previously lumped with *P. garrulus*, *P.* SP1, *P.* SP3, and *P.* SP2 due to morphological similarity with these species. *P. maculatus*, which occurs on consolidated soils, has several similarities (intermediate toe fringing, larger lepidosis, paired ovoid colour pattern, and intermediate body size) to *P.* SP3 which occurs on similar soils. Again, *P. kochi* and *P. garrulus*, both inhabiting loose sand, exhibit several similarities (extensive toe fringing, smaller lepidosis, spotted colour pattern) which are also visible in populations of *P.* SP1 and *P.* SP2 occurring in relatively loose sand. Some call traits which also appear to show convergence include call rate, with loose sand-living species which are not sisters, exhibiting a tendency for faster calls compared to their sister species on more consolidated soils. Non-sister species on consolidated soils tend to have longer notes and a more drawn-out first note compared to their sister species on loose soils. Additionally, the three coastal species (*P. carpi, P.* SP4 and *P.* SP1) living in the fog-zone exhibited several similar call characteristics (slow, low-pitched calls with low median call density and more variable inter-note intervals than other species), despite *P.* SP1 being distantly related to *P. carpi* and *P.* SP4

The centre of diversity of *Ptenopus* is in the Namib Desert, which contains six of the eight species while *P.* SP3 also occurs partially in the Namib Desert. It is therefore not unlikely that the genus originated in the central Namib Desert and thereafter diversified.

In keeping with the tradition of previous descriptions and taxonomic revisions of *Ptenopus,* this revision includes a major descriptive component of the advertisement calls, including a key. This is the most comprehensive description of its kind for any non-avian reptile. The advertisement call key is a useful tool for field identification of *Ptenopus*, as the calls are more easily detected and identified than the animals themselves.

CHAPTER 5: Synthesis, conclusions, and future directions for research on *Ptenopus*

Multiple lines of evidence including call data, phylogenetics, ecology, and morphology strongly support the existence of eight species within the genus *Ptenopus*, as opposed to the previously recognised three species. I revised the genus, describing the eight species as *P. kochi*, *P. carpi*, *P. garrulus*, *P. maculatus*, *P.* SP4*, P.* SP1, *P.* SP3, and *P. circumsytricus*, the last four having been newly named (although currecntly considered *nomen nudum*) in Chapter 4. While several of the species are morphologically similar, advertisement calls and the relative proportions of scales on the snout contain reliable diagnostic characters for this taxonomic model.

The central Namib Desert in western Namibia is the centre of diversity for *Ptenopus*. Five species, *P. kochi*, *P. carpi*, *P. maculatus*, and *P.* SP2 occur in this area and represent the most deeply divergent clades. *Ptenopus* SP1 also occurs in the southern Namib Desert, while *P.* SP3 occurs marginally in the Namib and in the pro-Namib. The Namib Desert also appears to be a site of active evolution, with multiple divergent clades present within several species, which appear to be ecologically divergent. *Ptenopus garrulus* is the most widespread species, occurring in xeric savannahs of the greater Kalahari and surrounding areas in Namibia, Botswana, South Africa, and southern Zimbabwe.

No genetic samples from Botswana, north-eastern South Africa or southern Zimbabwe were included in this study. However, this entire area is mainly covered with Arenosols (Fischer et al. 2008) and is thus somewhat similar to the Namibian Kalahari, which is a key habitat component for *P. garrulus* (Chapter 2). In addition, several recordings from iNaturalist and YouTube indicate a similar call of these populations to the Namibian Kalahari *P. garrulus* populations. This suggests that these populations which were not included in my study are most likely *P. garrulus*. However, considering the substantially higher rainfall and different ecoregions in the easternmost portions of this range (Sayre et al. 2013, Dinerstein et al. 2017), it is possible that these areas contain divergent clades or even additional species of *Ptenopus.* This would be revealed with further sampling from these areas.

There is phylogenetic support for more than the eight species divisions that I have described within *Ptenopus*. The species *P. maculatus, P. circumsytricus,* and *P.* SP1 have relatively deep intraspecific genetic divergences over relatively small geographic distances. The southernmost population of *P. garrulus* is almost certainly represents an additional species, but a thorough morphological and bioacoustic comparison is currently lacking. The populations of *Ptenopus garrulus*

in the southern Kalahari (southern Namibia and northern South Africa) also exhibit a complex spatial pattern of divergence, although these divergences are usually shallower. Generally, a higher spatial resolution of genetic samples is needed to properly assess several potential species-splits. Perhaps more importantly, a time-calibrated phylogeny of *Ptenopus* is needed to better evaluate the speciation process and the events that coincided with speciation. This will also provide a more comprehensive evaluation of the depth of genetic divergence between groups.

Several data-gaps prevented me from adequately assessing some of the putative species identified by the bGMYC species delimitation analyses (Chapter 3). Call recordings from many of these putative species are still lacking, and a thorough morphological comparison of some of these putative species has not yet been done. In particular, variation in hand and foot morphology and the toe fringes has been noticed among different populations within the described species. A quantification of these traits, which appear to be related to specialisation on different soils, may provide further insights into additional taxonomic divisions or speciation events in progress.

Divergent specialisation onto either soft sand or hard gravel has been instrumental in driving the speciation events described in my thesis, and several younger divergence events show a similar pattern. For instance, *P. circumsytricus* inhabits interdune plains, but not the dunes themselves. As a result, several populations live on small interdune "islands" separated by a sea of sand dunes which also move over time. This creates a dynamic mosaic of fragmented habitats that have undoubtedly led to some complex divergence patterns, as suggested by my data (see Appendix 2: Fig. S 4). Other species in the Namib may have been similarly affected by various patchy habitats, which may only be revealed by increased geographic sampling resolution. It is also possible that some major clades or even species in the Namib had not yet been genetically assessed. For instance, Haacke (1975) identified several morphologically anomalous "*Ptenopus garrulus*" populations around an isolated patch of dunes near the Brandberg, which were not included in my samples. Several interesting divergence patterns are also visible in *P. garrulus* in the Northern Cape, South Africa, which are possibly related to patchy substrates at the southern edge of the Kalahari Sand Sea. Higher geographic sampling resolution and fine-resolution soil maps may yet reveal additional speciation events in progress.

The burrow structures in *Ptenopus* is a subject in great need of further study. These burrows can be considered as an "extended phenotype" (Turner 2004) of *Ptenopus* species, which are likely acted on by intrinsic and extrinsic selective forces. Only qualitative descriptions of *Ptenopus* burrow structure have been made (e.g., Haacke 1975, this study). These observations suggest that burrow structure varies substantially among different species and genetic lineages of *Ptenopus,* which was previously interpreted merely as intraspecific geographic variation. Various features of the burrows

may serve diverse functions for these geckos in diverse environments. Observational notes (e.g., Haacke 1975, Polakow 1997, Hibbitts et al. 2005, 2012, Hibbitts 2006, Chapter 4) suggest substantial variation in the way that different species interact with their burrows. These include variation in traits such as regularity of burrow maintenance, entrance blocking and disguising, relative time spent in or outside the burrows, and cohabitation of burrows by different sexes and ages. The substrate specificity in *Ptenopus*, which my study found to be apparent in all species (Chapter 2), is likely influenced by the "phenotype" of burrows and vice versa.

Only two species of *Ptenopus* have been the subject of intentional ecological and natural history study: *P. kochi* (Polakow 1997) and *P. garrulus sensu stricto* (Hibbitts 2006). In light of the new species described, at least five species lack basic data on their life-history and ecology. Notes and observations on the various species (Chapter 4) suggest that clutch size, foraging behaviour, calling phenology, courtship displays, burrow maintenance and habitation traits, and several other ecological traits may vary among the species.

The analyses I presented on the advertisement calls strongly supported a mate recognition system in *Ptenopus*. Moreover, my analyses showed evidence of reproductive character displacement in call traits, which is preliminary evidence for the speciation by reinforcement hypothesis. This suggests that calls may play an important role in the speciation process of *Ptenopus*. However, I was unable to test female responses to male calls in behavioural experiments, which may add strong supporting evidence for the reinforcement hypothesis (e.g., Polakow 1997). Female preference of conspecific calls over heterospecific calls, and even calls from a native population compared to a distant population, need to be tested. In addition, complex vocal signals may include some call traits which are used for mate recognition and are under stabilising selection, whereas other traits are indicative of mate quality and may be under directional selection (e.g., Bailey et al. 2017). Principal component analysis (Chapter 3) identified some call traits which appear to be more different among the species, which are the likely candidates for traits under stabilising selection. Dominant frequency contributed less variation among species than call components related to rhythm. This trait may be more important as an indicator of animal size, possibly indicative of mate quality (see Hibbitts et al. 2007). Artificially generated calls could be used in female choice experiments to distinguish call components most important for female mate recognition vs. mate choice based on preference of mate quality.

Preliminary behavioural trials and observations suggest that the visual cues of a male calling are important to observing females, in addition to the audial cues. The bright yellow gular patches, in particular, warrant further investigation. The males in all species possess gular patches and in some cases, visibly display them to approaching females. Hence, these undoubtedly represent

important visual signals in courtship. All the gular patches are yellow, and it is worth noting that moonlight has strong spectral peaks in the yellow (~580 nm) spectrum (van der Steen 2014, Breitler et al. 2020), suggesting that the gular patches would be highly visible in moonlight. My observations suggest that most *Ptenopus* species synchronise their nocturnal calling activity with moon illuminance, with notable choruses starting after (later) moonrise and dramatic silences descending when the moon sets. There are variations in the shade of yellow and the size and shape of the gular patch among *Ptenopus* species, and several species also have yellow on the limbs and body (see species descriptions). These differences have not yet been quantified. The quantification of female perception of the gular patch colouration in natural settings (e.g., with only moonlight available, or with evening sunlight) may also be important. It is possible that gular patches or other visual elements of courtship may provide additional insight into mate recognition and speciation by reinforcement in *Ptenopus.*

One probable case of mate recognition based on gular patches is evident between *P. carpi* and *P.* SP4. *Ptenopus* SP4 females have no gular patch as in other species, while *P. carpi* females lack the gular patch. Contrary to the usual case where females respond to male signals (calls, gular patches), mate selection in this case may work in the opposite direction, since the difference between the clades is in the females. Behavioural experiments may confirm if *P.* SP4 males would react aggressively towards a female with a gular patch, mistaking it for a male. Conversely, *P. carpi* males from may not accept a female with the unusual condition of no gular patch.

Ptenopus proved to be an exceptionally interesting model for studying various speciation processes and evolutionary processes contributing to diversification. Evidence for various modes of speciation including divergent ecological specialisation, vicariance, and reinforcement, are provided by my data and observations. The range of unusual life-history traits of these geckos, including their calls, gular patches, burrow structure, and courtship interactions, may provide further insights into the evolutionary processes that contribute to the diversity of life on earth.

This thesis contributed to the recognition of several additional species to the Gekkonidae hotspot in xeric southern Africa, and at least two additional Namibian endemics, with the possibility of more species to be described. The study lays the foundation for further systematic revisions on the genus *Ptenopus*, which is clearly more speciose than had been appreciated over the last century.

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SUPPLEMENTARY MATERIALS

Appendix 1: Chapter 2 supplementary data and results

Table S 1: *Ptenopus* sequences included in this study with the inclusion of each gene (*16S, ND2* and *c-mos*) indicated as 'Y' = yes or 'N' = no, for each individual.

Taxon	Field no.	Country	Locality	Lat	Lon	*16S*	*ND2*	*c-mos*
P. carpi	FB382	Namibia	near Hoanib River	-19.356	13.147	Y	Y	Y
P. carpi	FB383	Namibia	near Hoanib River	-19.357	13.133	Y	Y	Y
P. carpi	FB378	Namibia	near Cape Cross, N of Henties Bay	-21.761	14.001	Y	Y	Y
P. carpi	FB373	Namibia	S of Omaruru River, Henties Bay	-22.158	14.309	Y	Y	Y
P. carpi	FB375	Namibia	N of Swakop Swakopmund	-22.618	14.538	Y	Y	Y
P. carpi	FBPtC36	Namibia	N of Swakop River, near Swakopmund	-22.665	14.576	Y	Y	Y
P. carpi	FBPtC33A	Namibia	S of Swakop River near Swakopmund	-22.694	14.584	Y	Y	Y
P. carpi	FBPtC33B	Namibia	S of Swakop River near Swakopmund	-22.694	14.584	Y	Y	N
P. carpi	FBPtC32C	Namibia	Walvis Bay, E of	-23	14.669	Y	Y	Y
P. carpi	FB357	Namibia	Walvis Bay Airport	-23.003	14.66	Y	Y	Y
P. carpi	FBPtC3A	Namibia	Walvis Bay, E of	-23.01	14.735	Y	Y	N
P. carpi	FBPtC3B	Namibia	Walvis Bay, E of	-23.01	14.735	Y	Y	Y
P. carpi	FBPtC3C	Namibia	Walvis Bay, E of	-23.01	14.735	Y	Y	N
P. carpi	FBPtC2	Namibia	18 km NW of Gobabeb	-23.432	14.947	Y	Y	N
P. carpi	FBPtC1	Namibia	18 km NW of Gobabeb	-23.433	14.946	Y	Y	N
P. g. garrulus	FB715	Namibia	Okondeka, Etosha National Park	-18.998	15.866	Y	Y	N

P. g. garrulus	FB361	Namibia	Omitara	-22.271	18.015	Y	Y	Y
P. g. garrulus	FB360B	Namibia	Omitara	-22.279	18.014	Y	Y	Y
P. g. garrulus	FB401	Namibia	40km SW of Buitepos	-22.575	19.735	Y	Y	Y
P. g. garrulus	FB657	Namibia	Windhoek, 40 km south of	-22.869	17.156	Y	Y	Y
P. g. garrulus	FB502	Namibia	Okongonga Guest Farm, Stampriet	-24.453	18.763	Y	Y	N
P. g. garrulus	FB589	Namibia	Farm Bloukop, Namibia	-25.095	19.85	Y	Y	Y
P. g. garrulus	FB643	Namibia	NE of Koës	-25.711	19.306	Y	Y	Y
P. g. garrulus	FB661	Namibia	NE of Koës	-25.711	19.306	Y	Y	Y
P. g. garrulus	RSP324	South Africa	Tswalu	-27.21	22.471	Y	Y	N
P. g. garrulus	RSP322	South Africa	Tswalu	-27.238	22.473	Y	Y	Y
P. g. garrulus	RSP325	South Africa	Tswalu	-27.24	22.474	Y	Y	N
P. g. garrulus	RSP313	South Africa	Tswalu	-27.3	22.454	Y	Y	N
P. g. garrulus	MB20953	South Africa	Groblersdorp	-28.835	22.573	Y	Y	N
P. g. garrulus	MB21582	South Africa	Kakamas	-28.839	20.286	Y	Y	Y
P. g. garrulus	MB21036	South Africa	Prieska	-29.835	22.578	N	Y	Y
P. g. garrulus	MB21038	South Africa	Prieska	-29.835	22.578	N	Y	Y
P. g. maculatus	FB698	Namibia	Sesfontein Lodge	-19.119	13.621	Y	Y	N
P. g. maculatus	FB390	Namibia	Hoanib River	-19.362	13.15	Y	Y	Y
P. g. maculatus	FB395	Namibia	Hoanib River	-19.363	13.15	Y	Y	Y
P. g. maculatus	FB551	Namibia	Spitzkoppe	-21.838	15.195	Y	Y	Y
P. g. maculatus	FB552	Namibia	Spitzkoppe	-21.838	15.195	Y	Y	Y
P. g. maculatus	FB566	Namibia	Spitzkoppe	-21.838	15.195	Y	Y	Y
P. g. maculatus	FB558	Namibia	Khan River, Karibib Distrit	-22.423	15.122	Y	Y	Y
P. g. maculatus	FB542	Namibia	farm Jakalswater, Karibib District	-22.595	15.375	Y	Y	Y
P. g. maculatus	FB544	Namibia	farm Jakalswater, Karibib District	-22.595	15.375	Y	Y	Y
P. g. maculatus	FBPtG1	Namibia	Walvis Bay, 48 km SE of	-23.184	14.927	Y	Y	N
P. g. maculatus	FB338B	Namibia	Mirabib Campsite	-23.468	15.359	Y	Y	Y

P. g. maculatus	FB337A	Namibia	Mirabib Campsite	-23.471	15.363	Y	Y	Y
P. g. maculatus	FB636	Namibia	Gobabeb	-23.561	15.039	Y	Y	Y
P. g. maculatus	FBPtX4	Namibia	Gobabeb	-23.567	15.037	Y	Y	N
P. g. maculatus	FBPtX1	Namibia	Gobabeb	-23.567	15.038	Y	Y	N
P. g. maculatus	FBPtX3	Namibia	Gobabeb	-23.567	15.038	Y	Y	N
P. g. maculatus	FBPtX2	Namibia	Gobabeb	-23.569	15.037	Y	Y	N
P. g. maculatus	FBPtG3	Namibia	SE of Gobabeb	-23.698	15.348	Y	Y	N
P. g. maculatus	FBPtX7	Namibia	Far East dunes, NW of Solitaire	-23.793	15.78	Y	Y	Y
P. g. maculatus	EI0404	Namibia	Solitaire	-24.177	15.979	Y	Y	N
P. g. maculatus	HL009	Namibia	Kulala Desert Lodge	-24.622	15.721	Y	Y	Y
P. g. maculatus	FB2023	Namibia	Keerwerder, NamibRand	-24.95	16.04	Y	Y	N
P. g. maculatus	FB593	Namibia	Koës	-25.936	19.085	Y	Y	Y
P. g. maculatus	FB334A	Namibia	Keetmanshoop	-26.55	18.167	Y	Y	Y
P. g. maculatus	FB333	Namibia	Keetmanshoop	-26.551	18.167	Y	Y	Y
P. g. maculatus	FB463	Namibia	farm Kuibis	-26.621	16.746	Y	Y	N
P. g. maculatus	FB454	Namibia	Aus district	-26.709	16.282	Y	Y	Y
P. g. maculatus	FB988	Namibia	Lüderitz	-26.736	15.095	Y	Y	Y
P. g. maculatus	FB2024	Namibia	Sperrgebiet, pump house along main road	-26.993	15.358	Y	Y	N
P. g. maculatus	NCP1616	Namibia	Kuboes	-28.468	16.865	Y	Y	N
P. g. maculatus	FT92	Namibia	Oranjemund Shepard’s Lodge	-28.545	16.497	Y	Y	N
P. g. maculatus	MB21564	South Africa	Onseepkans	-28.849	19.584	Y	Y	Y
P. g. maculatus	FB510	South Africa	McDougal's Bay	-29.292	16.881	Y	Y	Y
P. g. maculatus	FB512	South Africa	McDougal's Bay	-29.292	16.881	Y	Y	Y
P. g. maculatus	FB621	South Africa	Port Nolloth, 30 km inland of	-29.305	17.183	Y	Y	Y
P. g. maculatus	S630	South Africa	Beaufort West	-32.413	21.963	Y	Y	Y
P. g. maculatus	EI0306	South Africa	Beaufort West	-32.438	22.633	Y	Y	Y
P. g. maculatus	WC5641	South Africa	Rooidraai	-32.465	23.633	Y	Y	Y
P. g. maculatus	S136	South Africa	Prince Albert	-32.965	22.197	Y	Y	Y

P. g. maculatus	S137	South Africa	Prince Albert	-32.965	22.197	Y	Y	Y
P. g. maculatus	WC5599	South Africa	Glandale	-33.048	24.961	Y	Y	Y
P. kochi	FBPtK13	Namibia	NW of Gobabeb,	-23.359	14.817	Y	Y	Y
P. kochi	FBPtK11	Namibia	NW of Gobabeb,	-23.36	14.818	Y	Y	Y
P. kochi	FBPtK2	Namibia	Gobabeb	-23.56	15.037	Y	Y	Y
P. kochi	FB623	Namibia	Gobabeb	-23.561	15.038	N	Y	Y
P. kochi	FB444	Namibia	Far East dunes, NW of Solitaire	-23.681	15.647	Y	Y	Y
P. kochi	FB443	Namibia	Far East dunes, NW of Solitaire	-23.682	15.647	N	Y	Y

Appendix 2: Chapter 2 supplementary results (genetic-geographic connectivity maps)

There was considerably elevated divergence among the clades *P. g. maculatus* South, Central and East, particularly among the geographically closest samples (Figure S 1). There were negative residuals between one sample from *P. g. maculatus* South and some samples from *P. g. maculatus* Central, although the southern sample had missing portions of *ND2* sequence, making this estimation problematic.

Within clade *P. g. garrulus* (Figure S 2), elevated divergence across the Orange River was not clear, although the sample from Groblersdorp was genetically isolated from surrounding samples, separated by a long mountain range or rocky ridge in roughly the same location as the Orange River (panel A). The southern samples – a subclade containing only *P. g. maculatus* – were clearly genetically isolated from the rest of the clade across the Great Escarpment (panel B) although they largely occurred on intermediate soils (vs. mostly soft soils for the northern samples).

Within clade *P. g. maculatus* South (Figure S 3), some elevated divergence was evident across the Orange River. There were notably positive residuals between coastal and inland samples, indicating possible genetic isolation of these populations.

Within clade *P. g. maculatus* Central (Figure S 4), the northernmost sample on the gravel plains was notably genetically isolated from most other samples including those geographically closest. The westernmost sample from Gobabeb was also notably genetically isolated from the sample further east, whereas the north-eastern samples displayed notably negative residuals.

Within clade *P. g. maculatus* East (Figure S 5), the north-western and north-eastern samples showed nil or positive residuals, indicating likely genetic isolation across the Nama-group formation, but not across the Great Escarpment or the Orange River.

Within clade *P. g. maculatus* North (Figure S 6), there were notably positive residuals between samples of the southern subclade (primarily south of the Swakop River, with one sample in the Khan River), the geographically closest samples from the northern subclade (north of Khan River), and between the sample from Jakalswater (between the Swakop and Khan Rivers) and both other subclades.

Within clade *P. kochi* (Figure S 7), the samples north of the Kuiseb River were genetically isolated from those on the other side, both to the east and west – the latter two showing negative residuals.

Within clade *P. carpi* (Figure S 8), samples north and south of the Swakop River were genetically isolated from each other, although one sample from the northern subclade was south of the river.

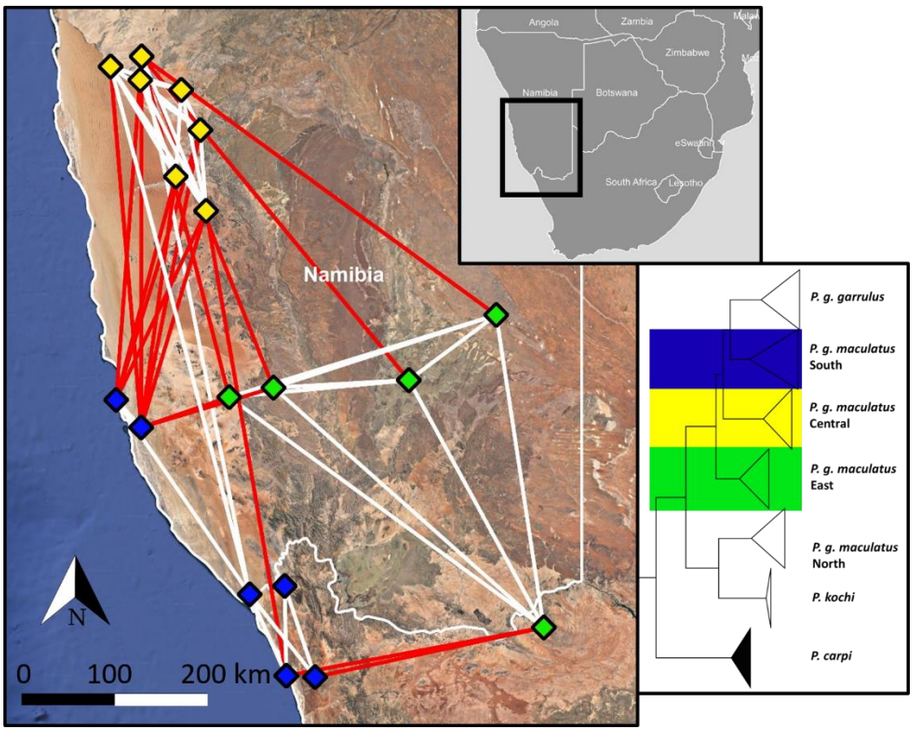

Figure S 1: Genetic-geographic connectivity map (p-distance residual SE = 0.025) of clades *P. g. maculatus* South, Central, and East (see summarised phylogeny on right panel) in South Africa and Namibia, showing positive residuals as red and negative residuals as white lines. Colours of samples on the map match those on the phylogeny extract on the right.

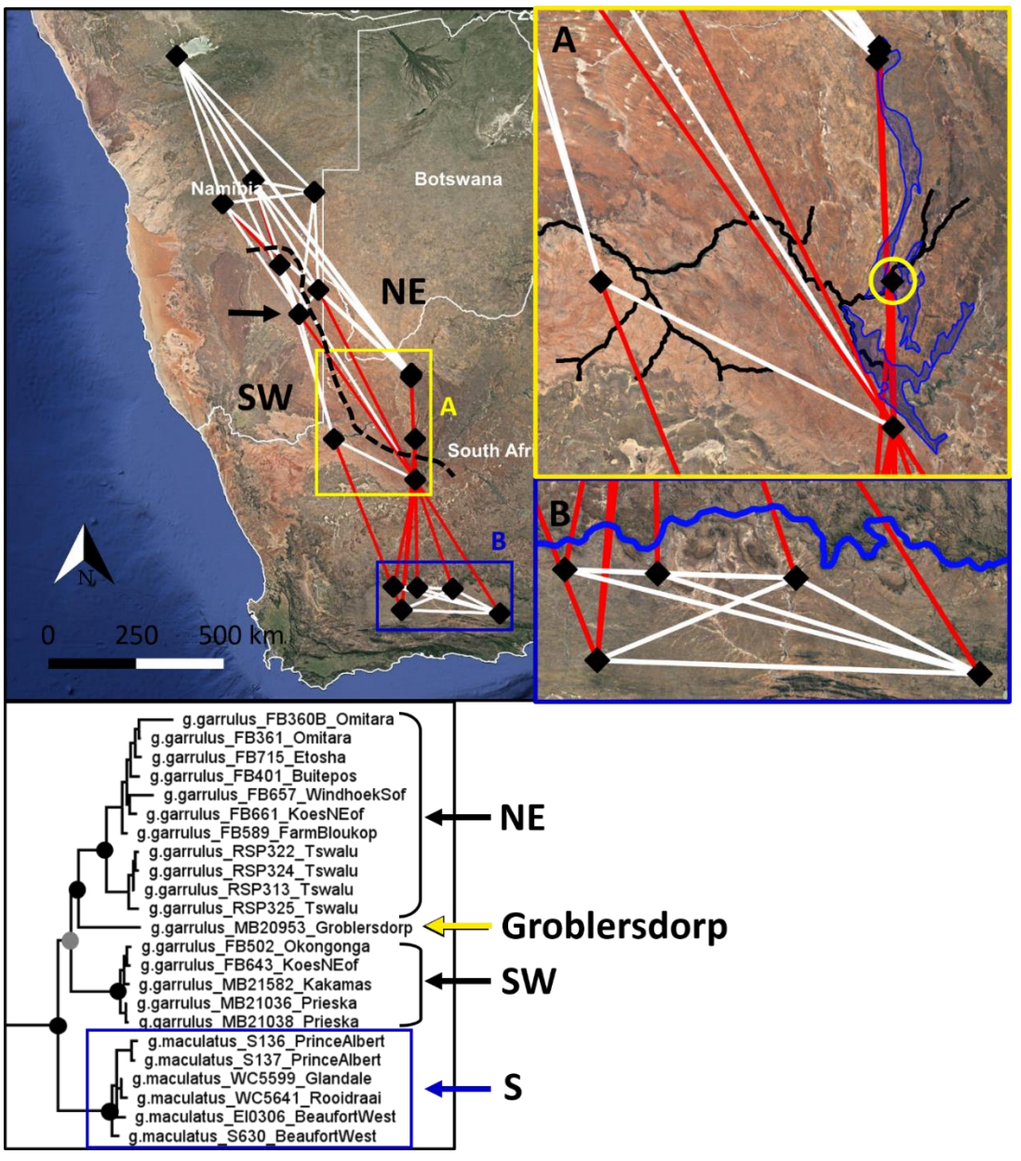

Figure S 2: Genetic-geographic connectivity map (p-distance residual SE = 0.022) of clade *P. g. garrulus* (see Figure 2) in South Africa and Namibia, showing positive residuals as red and negative residuals as white lines. A dotted line on the left panel roughly separates the south-western and north-eastern subclades, with one syntopic occurrence indicated by a black arrow. Enlarged panels on the right show potential geographic boundaries: rocky ridges (A) and the Great Escarpment (B), in blue. The bottom panel shows the *P. g. garrulus* clade extracted from the phylogeny, with the yellow arrow indicating the divergent sample from Groblersdorp, circled in yellow on map A, the blue arrow indicating a divergent clade shown in map B, and the samples northeast and southwest of the dotted line on the main map.

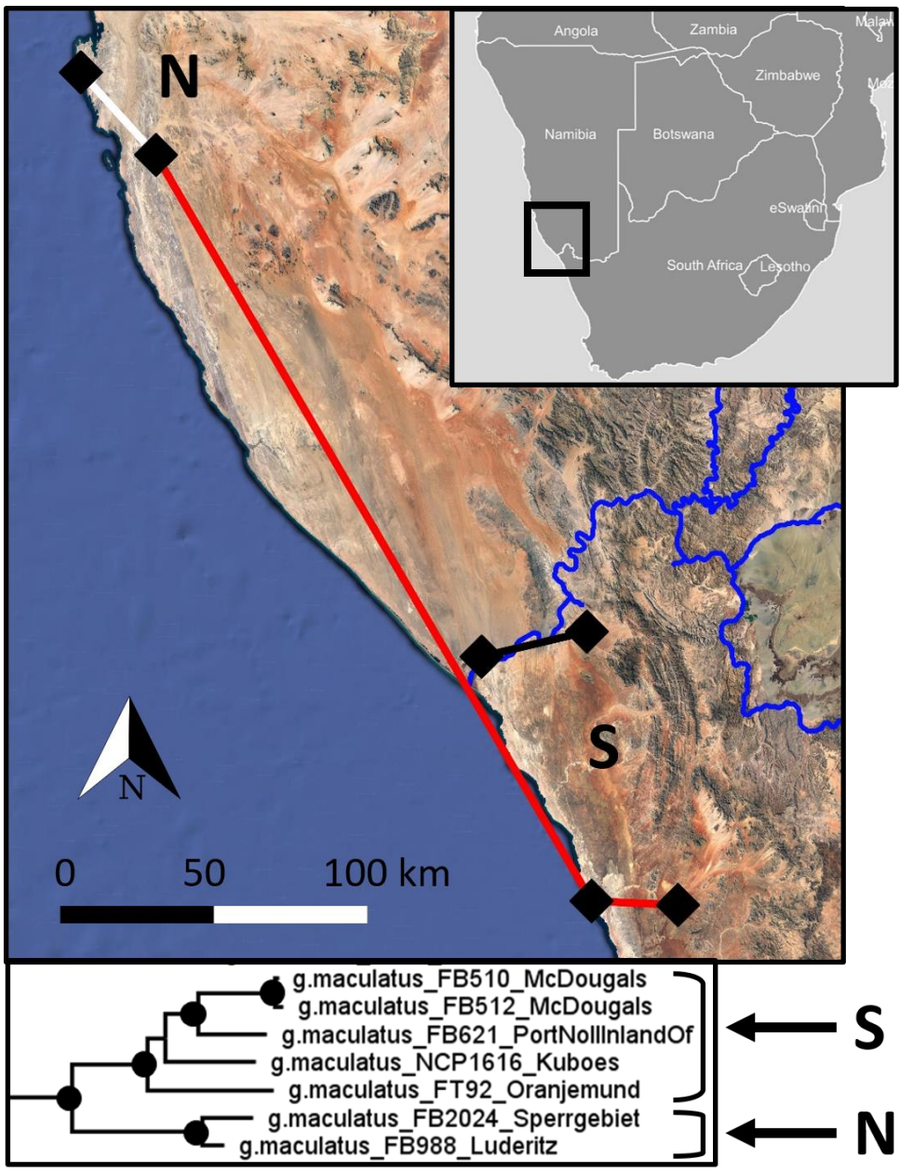

Figure S 3: Genetic-geographic connectivity map (R^2 = 0.85, p-distance residual SE = 0.0106) of clade *P. g. maculatus* South (see Figure 2) in southern Africa, showing positive residuals as red and negative residuals as white lines. Samples with expected relative divergence near the upper threshold (p-distance residual $\geq$ 0.01) indicated by black line. The Orange River is indicated by a jagged blue line. Northern and southern subclades are indicated on the map and phylogeny extract below.

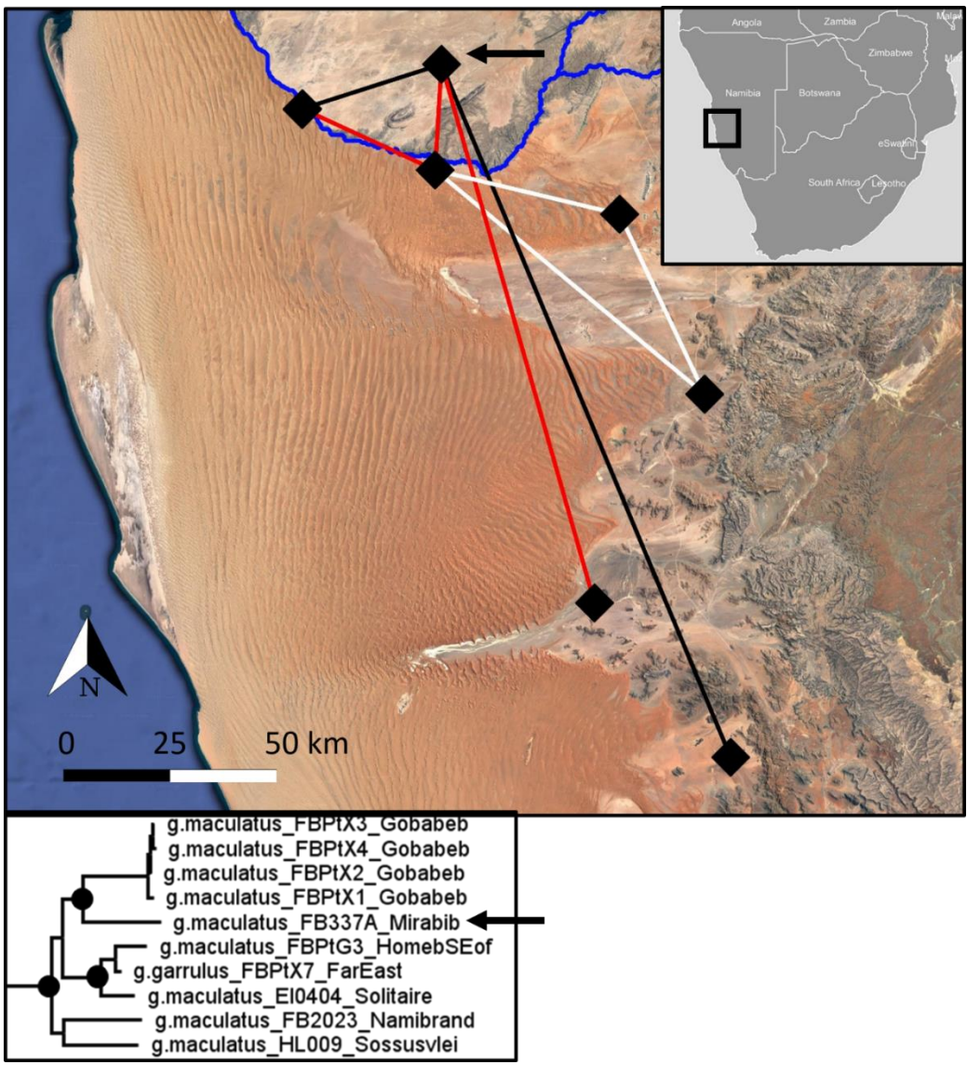

Figure S 4: Genetic-geographic connectivity map (R^2 = 0.76, p-distance residual SE = 0.0109) of clade *P. g. maculatus* Central (see Figure 2) in Namibia, showing positive residuals as red and negative residuals as white lines. Samples with expected relative divergence near the upper threshold (p-distance residual $\geq$ 0.0101) indicated by black lines. The Kuiseb River is indicated by a jagged blue line. One particularly divergent sample from the gravel plains is indicated by an arrow on the map and on the phylogeny extract below.

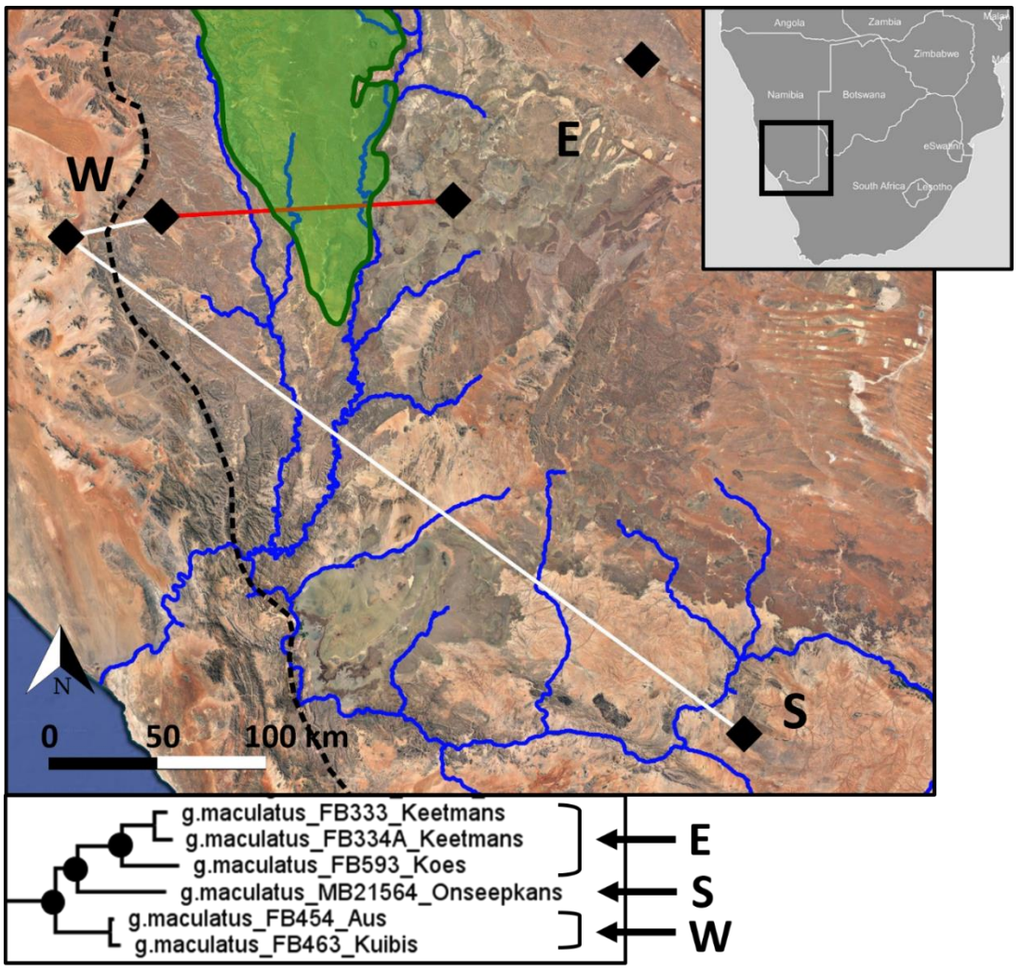

Figure S 5: Genetic-geographic connectivity map (R^2 = 0.70, p-distance residual SE = 0.0115) of clade *P. g. maculatus* East (see Figure 2) in southern Africa, showing positive residuals as red and negative residuals as white lines. The rocky Nama Group sedimentary formation is indicated in green, rivers (including the Orange River in the south) in blue, and the western escarpment by a black dotted line. Western, eastern, and southern samples are indicated on the map and the phylogeny extract below.

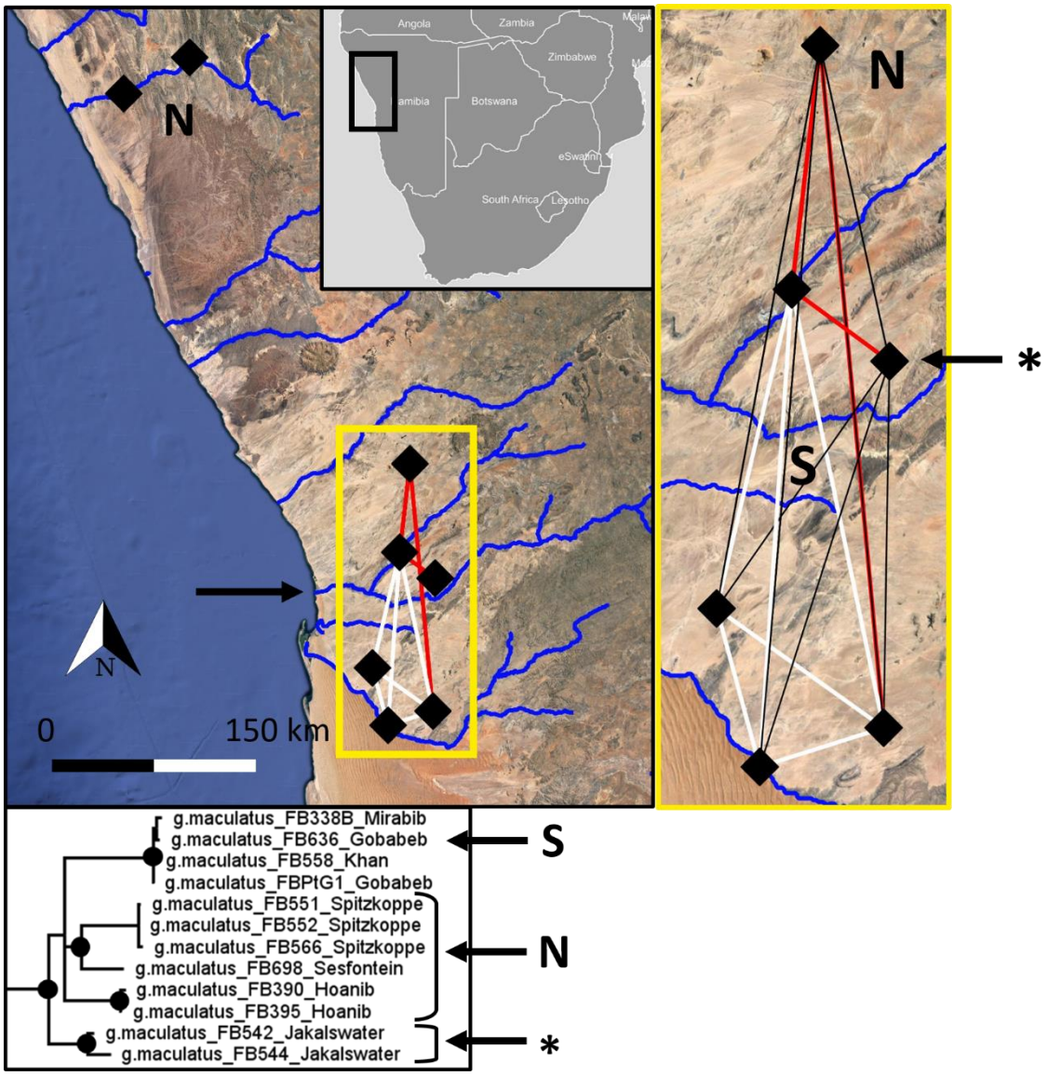

Figure S 6: Genetic-geographic connectivity map (R^2 = 0.37, p-distance residual SE = 0.0206) of clade *P. g. maculatus* North (see Figure 2) in Namibia, showing positive residuals as red and negative residuals as white lines. Rivers are indicated by blue lines, with the Swakop River mouth indicated on the left panel by a black arrow. The segment around the Swakop River and its northern tributary, the Khan River, is enlarged on the right panel, also showing expected relative sample divergence near the upper threshold (p-distance residual $\geq$ 0.012) as black lines. Samples from the northern and southern subclades and the one divergent subclade (*) from between the Swakop River and Khan Rivers, are indicated on the right-hand panel and the phylogeny extract below.

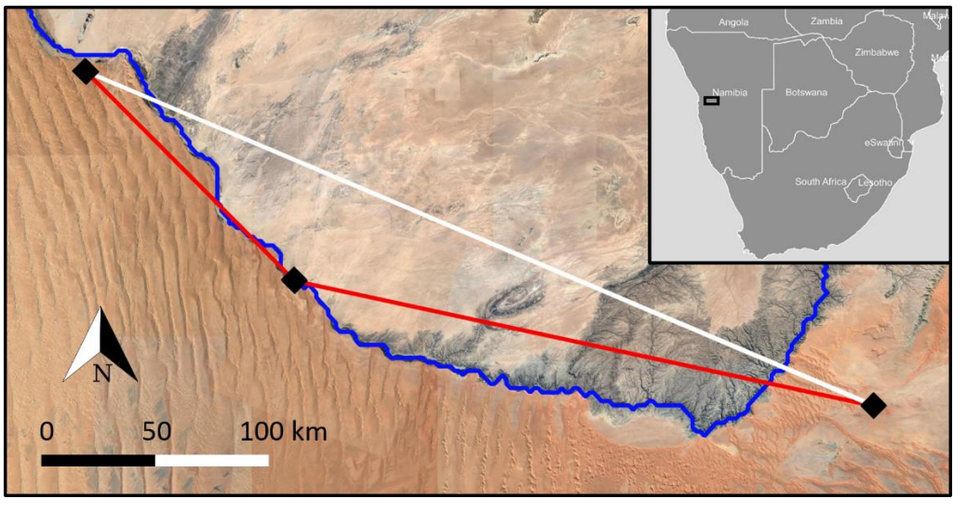

Figure S 7: Genetic-geographic connectivity map (R^2 = 0.20, p-distance residual SE = 0.0025) of clade *P. kochi* (see Figure 2) in Namibia, showing positive residuals as red and negative residuals as white lines; sample locations as black dots. Note the middle sample location is on the northern side of the Kuiseb River (intermediate soil), which is indicated by a black line.

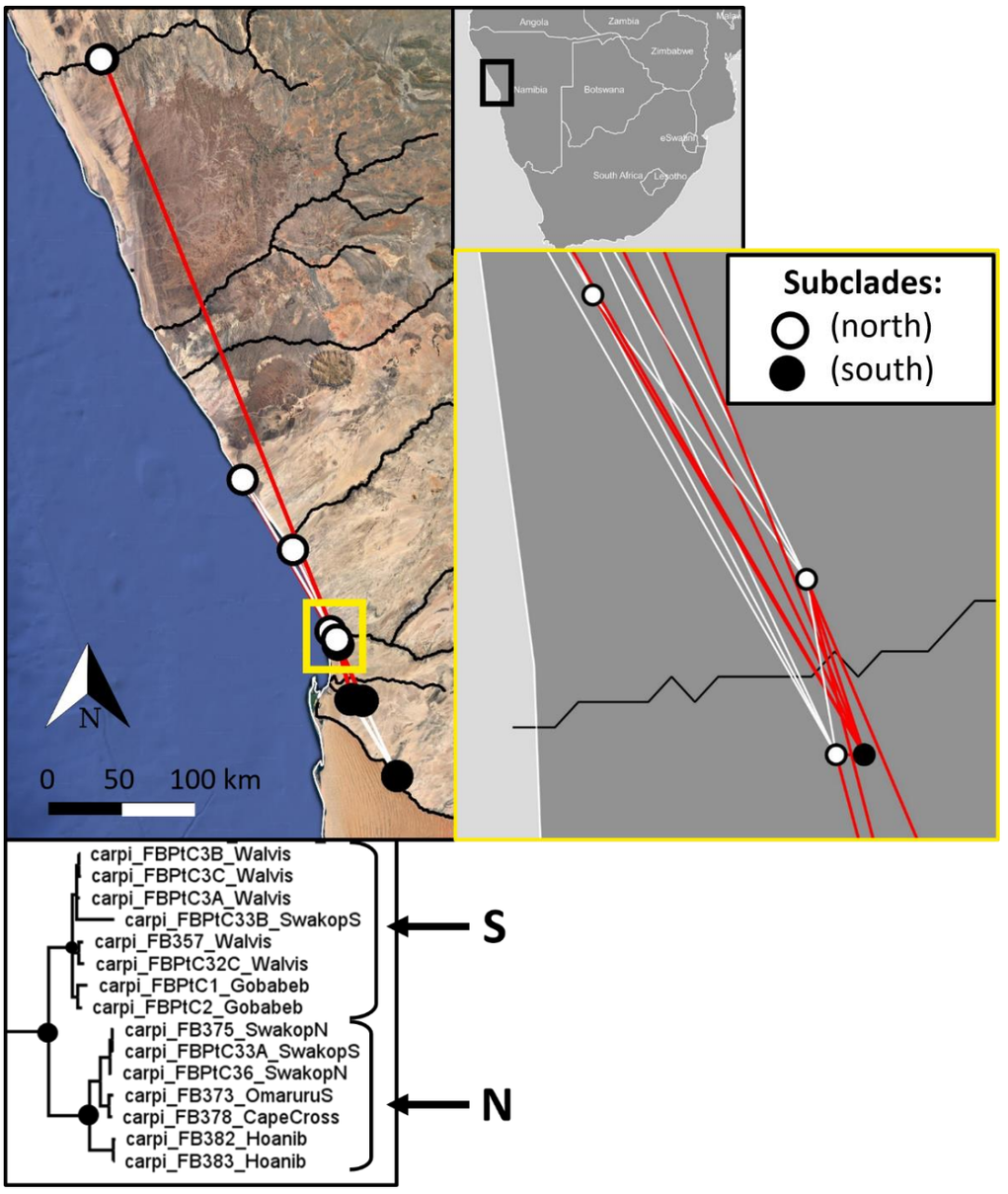

Figure S 8: Genetic-geographic connectivity map (R^2 = 0.22, p-distance residual SE = 0.0152) of clade *P. carpi* (see Figure 2) in Namibia, showing positive residuals as red and negative residuals as white lines. Rivers are indicated by black lines. The area around the Swakop River is enlarged on the right panel.

Appendix 3: Chapter 3 supplementary data and methods

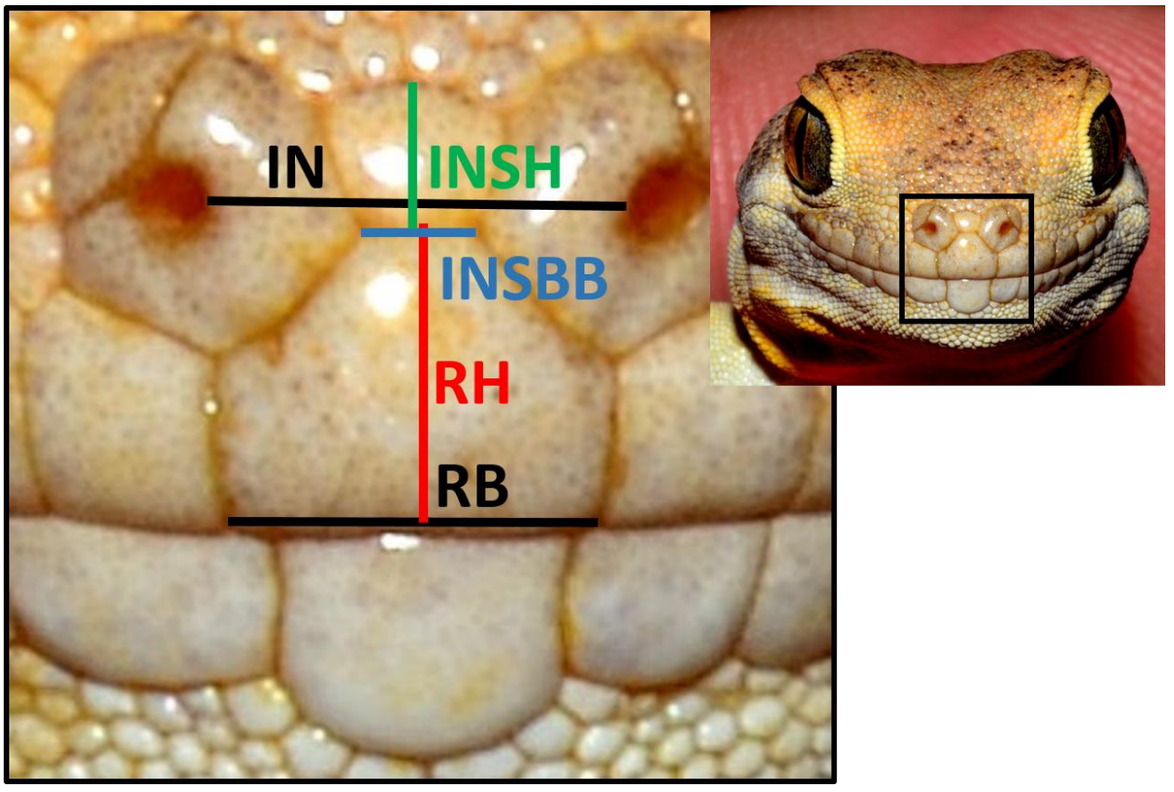

Figure S 9: Relative measurements taken on full frontal photographs of the snout of *Ptenopus* specimens for morphometric analysis.

Table S 2: Individual *Ptenopus* recorded for bioacoustic analyses, across the various putative species clades. Number of calls analysed per individual (n calls) are indicated.

Clade	Field no.	Country	Locality	Lat	Lon	Date	n calls
P. carpi A	FB854	Namibia	Carpi Wash, NW of Gobabeb	-23.482	14.981	2018-07-18	3
P. carpi A	FB865	Namibia	Carpi Wash, NW of Gobabeb	-23.482	14.981	2018-07-19	5
P. carpi A	FB869	Namibia	carpi wash, NW of Gobabeb	-23.481	14.978	2018-08-11	10
P. carpi B	FB1011	Namibia	near Hoanib River	-19.354	13.145	2019-05-13	10
P. carpi B	FB936	Namibia	near Hoanib River	-19.353	13.144	2019-05-13	2
P. carpi B	FB940	Namibia	near Hoanib River	-19.353	13.145	2019-05-13	7
P. carpi B	FB907	Namibia	Swakopmund area	-22.622	14.533	2018-10-08	1
P. carpi B	FB908	Namibia	Swakopmund area	-22.622	14.532	2018-10-08	4
P. carpi B	FB909	Namibia	Swakopmund area	-22.623	14.531	2018-10-08	5
P. carpi B	FB822	Namibia	Swakopmund, N of	-22.621	14.537	2018-05-13	1
P. g. garrulus	FB402	Namibia	40km SW of Buitepos	-22.575	19.735	2019-10-02	7
P. g. garrulus	FB953	Namibia	40km SW of Buitepos	-22.575	19.735	2019-10-02	9
P. g. garrulus	FB957	Namibia	40km SW of Buitepos	-22.338	19.753	2019-10-03	10
P. g. garrulus	FB1035	Namibia	Farm Bloukop	-25.097	19.849	2020-10-08	10
P. g. garrulus	FB594	Namibia	Farm Bloukop	-25.096	19.849	2020-10-08	10
P. g. garrulus	FB635	Namibia	Farm Bloukop	-25.097	19.849	2020-10-08	4
P. g. garrulus	FB640	Namibia	Farm Bloukop	-25.097	19.848	2020-10-08	10
P. g. garrulus	FB405	Namibia	Kalahari bush breaks	-22.336	19.753	2019-10-03	10
P. g. garrulus	FB408B	Namibia	Kalahari Bush Breaks farm, near Buitepos	-22.338	19.753	2019-10-03	10
P. g. garrulus	FB948	Namibia	Kalahari Bush Breaks farm, near Buitepos	-22.338	19.753	2019-10-01	10
P. g. garrulus	FB956	Namibia	Kalahari Bush Breaks farm, near Buitepos	-22.338	19.753	2019-10-03	10
P. g. garrulus	FB1031	Namibia	NE of Koës	-25.711	19.306	2020-10-07	10
P. g. garrulus	FB1033	Namibia	NE of Koës	-25.711	19.306	2020-10-07	10
P. g. garrulus	FB1039	Namibia	NE of Koës	-25.711	19.306	2020-10-07	6
P. g. garrulus	FB1041	Namibia	NE of Koës	-25.711	19.306	2020-10-07	4
P. g. garrulus	FB622	Namibia	NE of Koës	-25.711	19.306	2020-10-07	10
P. g. garrulus	FB414B	Namibia	Omitara	-22.280	18.013	2019-09-18	10
P. g. garrulus	FB360B	Namibia	Omitara area	-22.279	18.014	2019-09-18	10
P. g. garrulus	FB361	Namibia	Omitara area	-22.271	18.015	2019-09-18	10
P. g. garrulus	FB1036	Namibia	Windhoek, 40 km south of	-22.870	17.156	2020-11-02	10
P. g. garrulus	FB657	Namibia	Windhoek, 40 km south of	-22.869	17.156	2020-11-03	7
P. g. m. Central	FB744	Namibia	Gobabeb	-23.569	15.038	2017-09-07	17
P. g. m. Central	FB746	Namibia	Gobabeb	-23.567	15.038	2017-09-08	10

P. g. m. Central	FB747	Namibia	Gobabeb	-23.569	15.038	2017-09-22	10
P. g. m. Central	FB749	Namibia	Gobabeb	-23.569	15.038	2017-09-22	5
P. g. m. Central	FB878	Namibia	Gobabeb	-23.568	15.038	2018-08-13	10
P. g. m. Central	FBPtX2	Namibia	Gobabeb	-23.569	15.037	2017-09-10	10
P. g. m. Central	FBPtX3	Namibia	Gobabeb	-23.567	15.038	2017-09-12	10
P. g. m. Central	FB795	Namibia	Mirabib campsite	-23.455	15.382	2017-11-07	10
P. g. m. Central	FB797	Namibia	Mirabib campsite	-23.229	15.558	2017-11-09	7
P. g. m. East	FB958	Namibia	Aus area	-26.705	16.282	2019-11-21	10
P. g. m. East	FB959	Namibia	Farm Kuibis	-26.621	16.746	2019-11-24	12
P. g. m. East	FB902	Namibia	Keetmanshoop area	-26.550	18.167	2018-09-18	10
P. g. m. East	FB903	Namibia	Keetmanshoop area	-26.551	18.167	2018-09-18	10
P. g. m. East	FB904	Namibia	Keetmanshoop area	-26.551	18.167	2018-09-18	10
P. g. m. East	FB905	Namibia	Keetmanshoop area	-26.550	18.167	2018-09-18	9
P. g. m. East	FB1030	Namibia	Koës	-25.936	19.089	2020-10-06	7
P. g. m. East	FB608	Namibia	Koës	-25.937	19.089	2020-10-06	4
P. g. m. North	FB870	Namibia	carpi wash, NW of Gobabeb	-23.482	14.981	2018-08-11	7
P. g. m. North	FB885	Namibia	carpi wash, NW of Gobabeb	-23.482	14.979	2018-08-14	7
P. g. m. North	FB968	Namibia	farm Jakalswater, Karibib District	-22.591	15.370	2020-06-08	1
P. g. m. North	FB969	Namibia	Jakalswater, Karibib District	-22.589	15.370	2020-06-08	12
P. g. m. North	FB735	Namibia	Gobabeb	-23.561	15.039	2017-07-30	10
P. g. m. North	FB736	Namibia	Gobabeb	-23.562	15.039	2017-08-01	8
P. g. m. North	FB737	Namibia	Gobabeb	-23.562	15.039	2017-08-01	10
P. g. m. North	FB740	Namibia	Gobabeb	-23.561	15.039	2017-08-20	10
P. g. m. North	FB741	Namibia	Gobabeb	-23.562	15.040	2017-08-21	6
P. g. m. North	FB761	Namibia	Gobabeb	-23.562	15.040	2017-10-14	9
P. g. m. North	FB829A	Namibia	Gobabeb	-23.561	15.041	2018-06-12	10
P. g. m. North	FB830	Namibia	Gobabeb	-23.559	15.041	2018-06-12	10
P. g. m. North	FB831B	Namibia	Gobabeb	-23.561	15.039	2018-06-13	10
P. g. m. North	FB836A	Namibia	Gobabeb	-23.561	15.039	2018-06-16	10
P. g. m. North	FB986	Namibia	Gobabeb	-23.561	15.039	2017-07-30	10
P. g. m. North	FB926	Namibia	Hoanib River	-19.362	13.150	2019-05-11	4
P. g. m. North	FB930	Namibia	Hoanib River	-19.362	13.150	2019-05-12	11
P. g. m. North	FB931	Namibia	Hoanib River	-19.362	13.150	2019-05-12	10
P. g. m. North	FB932	Namibia	Hoanib River	-19.362	13.150	2019-05-12	12
P. g. m. North	FB933	Namibia	Hoanib River	-19.362	13.150	2019-05-12	5
P. g. m. North	FB913	Namibia	Mirabib	-23.472	15.364	2018-12-15	10
P. g. m. North	FB914	Namibia	Mirabib	-23.467	15.362	2018-12-15	10
P. g. m. North	FB916	Namibia	Mirabib	-23.468	15.360	2018-12-15	10
P. g. m. North	FB784	Namibia	Mirabib campsite	-23.469	15.364	2017-11-03	10
P. g. m. North	FB786	Namibia	Mirabib campsite	-23.473	15.364	2017-11-03	10
P. g. m. North	FB857A	Namibia	NW of Gobabeb	-23.482	14.980	2018-07-18	10
P. g. m. North	FB858A	Namibia	NW of Gobabeb	-23.482	14.980	2018-07-18	10

P. g. m. North	FB866	Namibia	NW of Gobabeb	-23.482	14.980	2018-07-19	10
P. g. m. South	FB1046	Namibia	Port Nolloth	-29.263	16.889	2020-11	10
P. g. m. South	FB979	South Africa	Port Nolloth	-29.270	16.912	2020-11-17	10
P. g. m. South	FB621	South Africa	Port Nolloth, 30 km inland of	-29.305	17.183	2020-11-19	10
P. g. m. South	FB982	South Africa	Port Nolloth, 30 km inland of	-29.305	17.183	2020-11-19	10
P. kochi	FB734	Namibia	Gobabeb	-23.561	15.039	2017-07-30	3
P. kochi	FB738	Namibia	Gobabeb	-23.562	15.040	2017-08-18	10
P. kochi	FB739	Namibia	Gobabeb	-23.562	15.040	2017-08-19	10
P. kochi	FB776	Namibia	Gobabeb	-23.565	15.037	2017-10-26	10
P. kochi	FB777	Namibia	Gobabeb	-23.565	15.037	2017-10-26	10
P. kochi	FB780	Namibia	Gobabeb	-23.562	15.040	2017-11-02	10
P. kochi	FB782	Namibia	Gobabeb	-23.562	15.040	2017-11-02	10
P. kochi	FB783	Namibia	Gobabeb	-23.562	15.040	2017-11-02	10
P. kochi	FB842	Namibia	Gobabeb	-23.565	15.037	2018-07-12	10
P. kochi	FB843	Namibia	Gobabeb	-23.566	15.037	2018-07-12	7
P. kochi	FB844b	Namibia	Gobabeb	-23.566	15.037	2018-07-12	10
P. kochi	FB844c	Namibia	Gobabeb	-23.566	15.037	2018-07-11	1
P. kochi	FB844	Namibia	Gobabeb	-23.566	15.037	2018-07-11	2
P. kochi	FB852	Namibia	Gobabeb	-23.562	15.040	2018-07-16	10
P. kochi	FB853	Namibia	Gobabeb	-23.562	15.040	2018-07-16	10
P. kochi	FB879	Namibia	Gobabeb	-23.566	15.036	2018-08-13	10
P. kochi	FB880	Namibia	Gobabeb	-23.566	15.037	2018-08-13	8
P. kochi	FBPtK3	Namibia	Homeb, SE of (Far East Dunes)	-23.795	15.783	2017-09-26	10

Code S 1: R code for analysing batches of calls, cut into separate WAV files. Details of various functions, packages, and parameters to set, are included in the extensive comments.

```
##############################################################################################
#########
##    ANALYSIS PER CALL
##############################################################################################
###

## Notes:
# same among all versions 0-4, except for a set of square brackets at "filPeaNot =", probably not
important
# NB: check you use version 1.8.2.tar.gz   2020-10-17 17:30            3.9M for
# 'soundgen' package! it changes a lot among versions and behaves inconsistently

#################################################################
##Packages:
require(soundgen)
require(seewave)
require(tuneR)

##############################################################################################
#
## CHANGE FILE-SPECIFIC PARAMETERS IN FOLLOWING SECTION:

## Set the location of the files to be analysed and make list of the files
setwd("C:/Users/user/Documents/Ptenopus/Data programs other/Call analyses/extracts 4")

## new list of file names, only .wav / .WAV
fileName = list.files()
fileName = fileName[(grepl(".WAV",fileName))|(grepl(".wav",fileName))]

## recording ID: file name without _# or extension
recordID = lapply(fileName, function(x){
  gsub("_.*", "", x)
})

## call Nr: # after the '_' in the file name (probably not the most efficient way)
calNr = lapply(fileName, function(x){
  gsub(".*_", "", x)
})
# remove extension
calNr = lapply(calNr, function(x){
  gsub("(.*)\\..*$", "\\1", x)
})
# make numeric
calNr = as.numeric(unlist(calNr))

## call ID: file name without extension
calID = lapply(fileName, function(x){
  gsub("(.*)\\..*$", "\\1", x)
})

##############################################################################################
#########
##  convert Wav files to Wave objects (TuneR)

waves = lapply(fileName, function(x){
  readWave(x)
})

##############################################################################################
#########
## Frequency analysis of whole call
```

```
#############################################################################################
#########

## list of peaks for power spectrum for each l
frePea = lapply(waves, function(x){
  fpeaks(meanspec(x, wl = 1024, ovlp = 50, plot =  F),
         plot = F)
})

## dominant freqcy
domFre = lapply(frePea, function(x){
  x[,1][x[,2] == max(x[,2])]
})

## dom freq amplitude
domFreAmp = lapply(frePea, function(x){
  x[,2][x[,2] == max(x[,2])]
})

## basal frequency: won't be below 300 Hz
basFre = lapply(frePea, function(x){
  min(x[,1][x[,1] >= 0.3])
})

# basal freq amp
basFreAmp = lapply(frePea, function(x){
  min(x[,2][x[,1] >= 0.3])
})

# basal freq amplitude % reduction from domFreq
basFreAmpRed = lapply(1:length(fileName), function(x){
  (domFreAmp[[x]]-basFreAmp[[x]]) / domFreAmp[[x]]*100
})

## lower 90% bandwidth peak
banWidLow = lapply(frePea, function(x){
  min(x[,1][
    x[,2] >= (0.1*(x[,2][x[,2] == max(x[,2])]))
    ])
})

## upper 90% bandwidth peak
banWidUpp = lapply(frePea, function(x){
  max(x[,1][
    x[,2] >= (0.1*(x[,2][x[,2] == max(x[,2])]))
    ])
})


#####################################################################
## 2nd emphasised freqcy:
## if > 1kHz different from dom freq and < 6 kHz
## (and its amplitude):

# filter step
filPea = lapply(1:length(fileName), function(x){
  frePea[[x]][
    frePea[[x]][,1] < 6 &
      frePea[[x]][,1] > (domFre[[x]]+1) |
      frePea[[x]][,1] < (domFre[[x]]-1)
    ,,drop = F]
})
```

```
# 2nd emphasised freq
empFre2 = lapply(filPea, function(x){
  x[,1][x[,2] == max(x[,2])]
})

# 2nd emph freq amplitude
empFre2Amp = lapply(filPea, function(x){
  x[,2][x[,2] == max(x[,2])]
})

# 2nd freq amplitude % reduction from domFreq
empFre2AmpRed = lapply(1:length(fileName), function(x){
  (domFreAmp[[x]]-empFre2Amp[[x]]) / domFreAmp[[x]]*100
})

##############################################################################################
#########
## Segment call into notes for further analysis
##############################################################################################
#########


##############################################################################################
#########
## Filer out noise and ready the calls for segmentation

## filter out frequencies below 0.9 kHz (mostly noise)
## converting wav to vector
wavesF = lapply(waves, function(x){
  ffilter(x, from = 0, to = 900, bandpass = F,
          wl = 1024, ovlp = 50,
          output = "matrix")
})

##  calculate the amplitudes for the frequency-filtered noise for time
amplts = lapply(1:length(wavesF), function(x){
  env(wavesF[[x]], f = waves[[x]]@samp.rate, envt = "abs", msmooth = c(32, 50),
      norm = F, plot = F)
})

##############################################################################################
#########
##  set the syllable threshold value at a standard, normalised to the loudest 2%
##    of the call (rather than normalising to the mean amplitude of the whole sound,
##    which is the default for function "segment" below, and causes too much vairability)

# scaling factor: mean amplitude of top 1%
SF = lapply(amplts, function(x)
{mean(na.omit(tail(sort(x), (nrow(x)/100))))})


# syllable threshold for segmentation ("segment" below), normalising
# mean amplitude to SF:
ST = lapply(1:(length(fileName)), function(x){
  SF[[x]]/20/mean(amplts[[x]], na.rm = T)
})


##############################################################################################
#########
##  Segment the sound into syllables using normalised threshold value (ST), for
##  initial note break-up times
```

```
seg = lapply(1:(length(fileName)), function(x){
  segment(wavesF[[x]][,1], samplingRate = waves[[x]]@samp.rate,
          windowLength = (3=2/(waves[[x]]@samp.rate/1000)),
          overlap = 50, shortestSyl = 1,
          sylThres = ST[[x]], shortestPause = 15,# interburst = 50,
          burstThres = 1,
          plot = T, main = fileName[[x]]
  )})

## Filter the segments to only syllables (remove bursts) > 15 MS,
## to cut out remaining noise bursts

seg = lapply(seg, function(x){
  x$syllables[(x$syllables$sylLen > 15),]
})


#########################################################################################
################################
## Frequency analyses per note
#########################################################################################
#########


## At sampling rate of x kHz, a single sample lasts MS milliseconds,
## hence the first sample is from 0 to MS milliseconds, the second from
## MS to MS*2 milliseconds, etc:
MS = lapply(1:length(fileName), function(x){
  1/(waves[[x]]@samp.rate/1000)
})


## list of peaks for spectograms for each note, within each call
frePeaNot = lapply(1:length(fileName), function(x){
  lapply(1:nrow(seg[[x]]), function(y){
    fpeaks(meanspec(
      waves[[x]][
        (if((seg[[x]]$start[y]/MS[[x]]-15/MS[[x]])<=0){
          1}else{(seg[[x]]$start[y]/MS[[x]]-15/MS[[x]])}): #the sample number, e.g., 4th sample, that
starts at that particular time in milliseconds, (should actually be seg[[x]]$start[1])/MS+1), but we'll
start ~15 milliseconds early and end 10 ms late just to give time for an adequate time analysis; built-
in safety against starting before the start of the recording
          (seg[[x]]$end[y]/MS[[x]]+15/MS[[x]])],
      wl = 1024, ovlp = 50, plot =  F),
      plot = F)
  })
})

## dominant freqcy (per note per call)
domFreNot = lapply(1:length(fileName), function(x){
  sapply(1:nrow(seg[[x]]), function(y){
    frePeaNot[[x]][[y]][,1][
      frePeaNot[[x]][[y]][,2] ==
        max(frePeaNot[[x]][[y]][,2])
      ]
  })
})

## basal frequency: won't be below 350 Hz
basFreNot = lapply(1:length(fileName), function(x){
  lapply(1:nrow(seg[[x]]), function(y){
    min(frePeaNot[[x]][[y]][,1][
      frePeaNot[[x]][[y]][,1] >= 0.35
```

```
    ])
  })
})

#####################################################################
## 2nd emphasised freqcy (per note per call):
## if > 1kHz different from dom freq and < 6 kHz
## (and its amplitude):

# filter step
filPeaNot = lapply(1:length(fileName), function(x){
  lapply(1:nrow(seg[[x]]), function(y){
    frePeaNot[[x]][[y]][
      frePeaNot[[x]][[y]][,1] < 6 &
        frePeaNot[[x]][[y]][,1] > (domFreNot[[x]][[y]]+1) |
        frePeaNot[[x]][[y]][,1] < (domFreNot[[x]][[y]]-1)
      ,,drop = F]

  })
})


# 2nd emphasised freq
empFre2Not = lapply(1:length(fileName), function(x){
  lapply(1:nrow(seg[[x]]), function(y){
    filPeaNot[[x]][[y]][,1][
      filPeaNot[[x]][[y]][,2] == max(filPeaNot[[x]][[y]][,2])
      ]
  })
})


#############################################################################################
#################
## Condense per note frequency measures to reported variables
#############################################################################################
###########

## median dominant frequency for all notes
domFreNotMd = lapply(domFreNot, function(x){
  median(x)})

## range of dominant frequency for all notes
domFreNotRan = lapply(domFreNot, function(x){
  max(x)-min(x)})

## first note dominant frequency deviance
domFre1Dev = lapply(domFreNot, function(x){
  x[1]-median(x)
})


#############################################################################################
#########
##  further variable columns for analysis (some already done above)
#############################################################################################
#########


#############################################################################################
#########
##note/call timing variables:
```

```
#number of notes:
nNot = lapply(seg, function(x){nrow(x)})

#call duration
calDur = lapply(seg, function(x){
  (x$end[nrow(x)] - x$start[1])/1000
})

#note rate (/second):
notRat = lapply(1:(length(fileName)), function(x){
  (nNot[[x]]-1)/((seg[[x]]$start[nNot[[x]]]-seg[[x]]$start[1])/1000)})

#median note duration (milliseconds):
notDurMd = lapply(seg, function(x){median(x$sylLen)
})

#note duration range %
notDurRan = lapply(seg, function(x){
  (max(x$sylLen) - min(x$sylLen)) / median(x$sylLen) * 100
})

##first note duration deviance as percentage:
notDur1Dev = lapply(1:length(seg), function(x){
  (seg[[x]]$sylLen[1] - notDurMd[[x]]) / notDurMd[[x]]*100
})

#longest note number
lonNotNr = lapply(seg, function(x){
  mean(x$syllable[x$sylLen==max(x$sylLen)])})

#shortest note number
shoNotNr = lapply(seg, function(x){
  mean(x$syllable[x$sylLen==min(x$sylLen)])})


##############################################################################################
#########
##inter-note timing analysis:

#median Inter-note interval (milliseconds):
intNotIntMd = lapply(seg, function(x){median(x$pauseLen, na.rm = T)})

#inter-note interval range percentage:
intNotIntRan = lapply(1:length(seg), function(x){
  (max(seg[[x]]$pauseLen, na.rm = T) - min(seg[[x]]$pauseLen, na.rm = T)) /
    (intNotIntMd[[x]])*100
})

#longest inter-note number
lonIntNotNr = lapply(seg, function(x){
  x$syllable[x$pauseLen==max(x$pauseLen, na.rm = T)][1]
})

#shortest inter-note number
shoIntNotNr = lapply(seg, function(x){
  x$syllable[x$pauseLen==min(x$pauseLen, na.rm = T)][1]
})

##############################################################################################
#########
#  save data as CSV file

## put all relevant variables in order into a data.frame, taking care to convert frequencies to Hz
```

```
df = cbind(recordID, calNr, "calStaTim" = rep("", length(fileName)),
        "domFre"=unlist(domFre)*1000, "basFre"= unlist(basFre)*1000, basFreAmpRed,
        "empFre2"= unlist(empFre2)*1000, empFre2AmpRed,
        "banWidLow"=unlist(banWidLow)*1000,"banWidUpp"=unlist(banWidUpp)*1000,
        "domFreNotMd" =unlist(domFreNotMd)*1000,"domFreNotRan"
=unlist(domFreNotRan)*1000,
        "domFre1Dev"=unlist(domFre1Dev)*1000,
        nNot, calDur, notRat, notDurMd, notDurRan, notDur1Dev, lonNotNr, shoNotNr,
        intNotIntMd, intNotIntRan, lonIntNotNr, shoIntNotNr)


################################################################################
#########
#  extra information

## list of field names
fieldNames = colnames(df)

## information on field names
fieldInformation = c("recording ID", "call number", "call start time (min)",
                "dominant frequency (Hz)", "basal frequency (Hz)",
                "basal frequenct ampluitude reduction (% of domFre)",
                "2nd emphasised frequency (Hz)",
                "2nd emph freq amplitude reduction (% of domFre)",
                "lower 90% bandwidth peak (Hz)",
                "upper 90% bandwidth peak (Hz)",
                "dominant frequency median among notes (Hz)",
                "range (max-min) of dominant frequency (Hz) among notes",
                "1st note deviance in dominant frequency (Hz)",
                "number of notes", "call duration (s)",
                "note rate (notes/s)",
                "median note duration (ms)",
                "range in note duration (% of median)",
                "1st note deviance in duration (% of median)",
                "longest note nr",
                "shortest note nr",
                "inter-note interval median (ms)",
                "inter-note interval range (% of median)",
                "longest inter-note interval nr",
                "shortest inter-note interval nr")

df2 = cbind(fieldNames,fieldInformation)

require(qpcR)

## BIND THE two data frames, filling blanks with NA
df3 = qpcR:::cbind.na(df,df2)

## replace NA with ""
df3[is.na(df3)] = ""

##save as CSV
write.csv(df3, paste0(getwd(),"/metadata per call.csv"), row.names = F)

################################################################################
##write additional per note metadata for later use

## metadata per note
write.csv(cbind("recCallID" = unlist(lapply(1:length(seg), function(x){
  rep(callID[[x]], nrow(seg[[x]]))
})),
"noteNr" = unlist(lapply(seg, function(x){
  1:nrow(x)
```

```
})),
"domFreNot" = unlist(domFreNot),
"basFreNot" = unlist(basFreNot),
"empFre2Not" = unlist(empFre2Not),
"notDur" = unlist(lapply(seg, function(x){
  x$sylLen
})),
"intNotDur" = unlist(lapply(seg, function(x){
  x$pauseLen
}))),
paste0(getwd(),"/metadata per note.csv"), row.names = F, quote = F)

##############################################################################################
####
## clear the memory
rm(list = ls())
```

Appendix 4: Chapter 3 supplementary results

Automatic barcode gap detection (ABGD) results

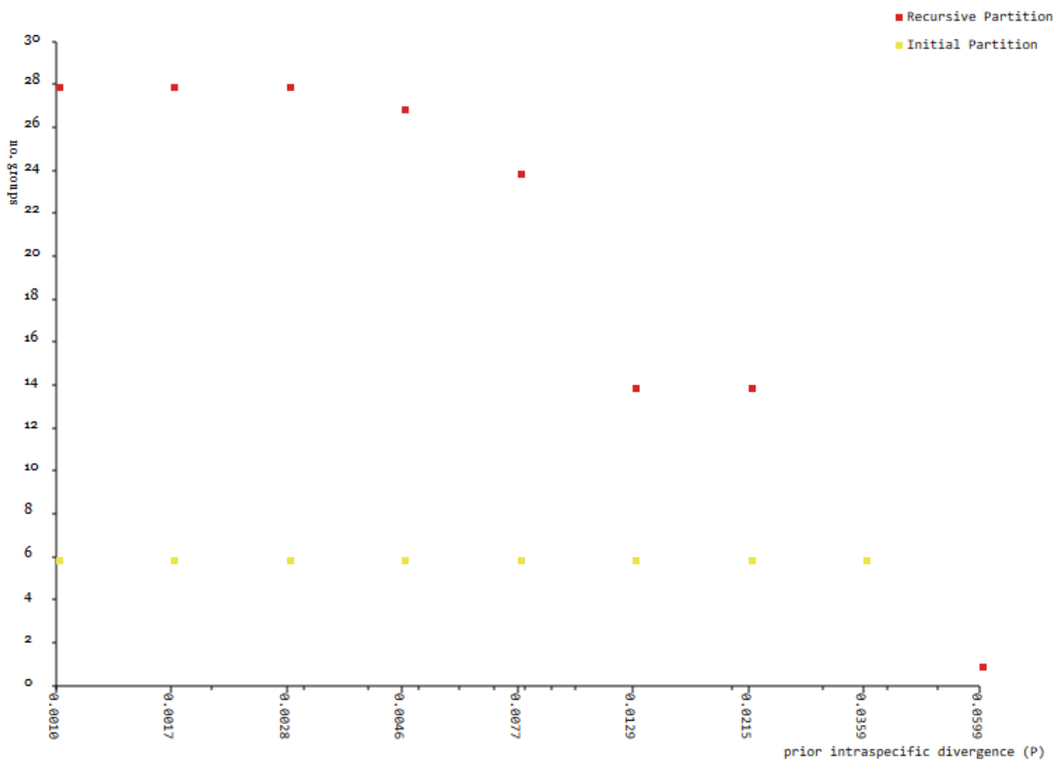

Figure S 10: Number of groups identified by ABGD Web Application at various prior intraspecific p-distances (in proportion), with initial partitions in yellow and recursive partitions in red. The groupings from the initial partitions are indicated on the phylogeny in Fig. 4.

List S 1: Samples within groups identified by the recursive partitions with 28 (maximum) groups

Note: Below are the sample numbers and names as shown in the phylogeny (Fig. 4) and in Appendix 1: Table S1, listed and space-separated per group, for each group identified in the maximum number of groups identified in the recursive partitions (28 groups; prior maximal distance for recursive partition P=1.00e-03).

Group[1] n: 7 ;id: carpi_FB357_Walvis carpi_FBPtC1_Gobabeb carpi_FBPtC2_Gobabeb carpi_FBPtC3A_Gobabeb carpi_FBPtC3B_Gobabeb carpi_FBPtC3C_Gobabeb carpi_FBPtC32C_Walvis

Group[2] n: 6 ;id: g.maculatus_EI0306_BeaufortWest g.maculatus_S136_PrinceAlbert g.maculatus_S137_PrinceAlbert g.maculatus_S630_BeaufortWest g.maculatus_WC5599_Glandale g.maculatus_WC5641_Rooidraai

Group[3] n: 3 ;id: g.maculatus_EI0404_Solitaire g.maculatus_FBPtG3_HomebSEof g.maculatus_FBPtX7_FarEast

Group[4] n: 6 ;id: g.maculatus_FB333_Keetmans g.maculatus_FB334A_Keetmans g.maculatus_FB454_Aus g.maculatus_FB463_Kuibis g.maculatus_FB593_Koës g.maculatus_MB21564_Onseepkans

Group[5] n: 4 ;id: g.maculatus_FB338B_Mirabib g.maculatus_FB558_Khan g.maculatus_FB636_Gobabeb g.maculatus_FBPtG1_Gobabeb

Group[6] n: 6 ;id: kochi_FB443_FarEast kochi_FB444_FarEast kochi_FB623_Gobabeb kochi_FBPtK2_Gobabeb kochi_FBPtK11_GobabebNWof kochi_FBPtK13_GobabebNWof

Group[7] n: 2 ;id: carpi_FB373_OmaruruS carpi_FB378_CapeCross

Group[8] n: 2 ;id: carpi_FB382_Hoanib carpi_FB383_Hoanib

Group[9] n: 1 ;id: carpi_FBPtC33B_SwakopS

Group[10] n: 7 ;id: g.maculatus_FB360B_Omitara g.maculatus_FB361_Omitara g.maculatus_FB401_Buitepos g.maculatus_FB589_FarmBloukop g.maculatus_FB657_WindhoekSof g.maculatus_FB661_KoësNEof g.maculatus_FB715_Etosha

Group[11] n: 5 ;id: g.maculatus_FB502_Okongonga g.maculatus_FB643_KoësNEof g.maculatus_MB21036_Prieska g.maculatus_MB21038_Prieska g.maculatus_MB21582_Kakamas

Group[12] n: 2 ;id: g.maculatus_FB510_McDougals g.maculatus_FB512_McDougals

Group[13] n: 1 ;id: g.maculatus_FB621_PortNollothInlandOf

Group[14] n: 1 ;id: g.maculatus_FB988_Lüderitz

Group[15] n: 1 ;id: g.maculatus_FB2024_Sperrgebiet

Group[16] n: 1 ;id: g.maculatus_FT92_Oranjemund

Group[17] n: 1 ;id: g.garrulus_MB20953_Groblersdorp

Group[18] n: 1 ;id: g.maculatus_NCP16_16_Richtersveld

Group[19] n: 4 ;id: g.garrulus_RSP313_Tswalu g.garrulus_RSP322_Tswalu g.garrulus_RSP324_Tswalu g.garrulus_RSP325_Tswalu

Group[20] n: 1 ;id: g.maculatus_FB337A_Mirabib

Group[21] n: 1 ;id: g.maculatus_FB2023_Namibrand

Group[22] n: 4 ;id: g.maculatus_FBPtX1_Gobabeb g.maculatus_FBPtX2_Gobabeb g.maculatus_FBPtX3_Gobabeb g.maculatus_FBPtX4_Gobabeb

Group[23] n: 1 ;id: g.maculatus_HL009_Sossusvlei

Group[24] n: 2 ;id: g.maculatus_FB390_Hoanib g.maculatus_FB395_Hoanib

Group[25] n: 2 ;id: g.maculatus_FB542_Jakalswater g.maculatus_FB544_Jakalswater

Group[26] n: 3 ;id: g.maculatus_FB551_Spitzkoppe g.maculatus_FB552_Spitzkoppe g.maculatus_FB566_Spitzkoppe

Group[27] n: 1 ;id: g.maculatus_FB698_Sesfontein

Group[28] n: 3 ;id: carpi_FB375_SwakopN carpi_FBPtC33A_SwakopS carpi_FBPtC36_SwakopN

Bayesian general mixed Yule-coalescent model (bGMYC) results

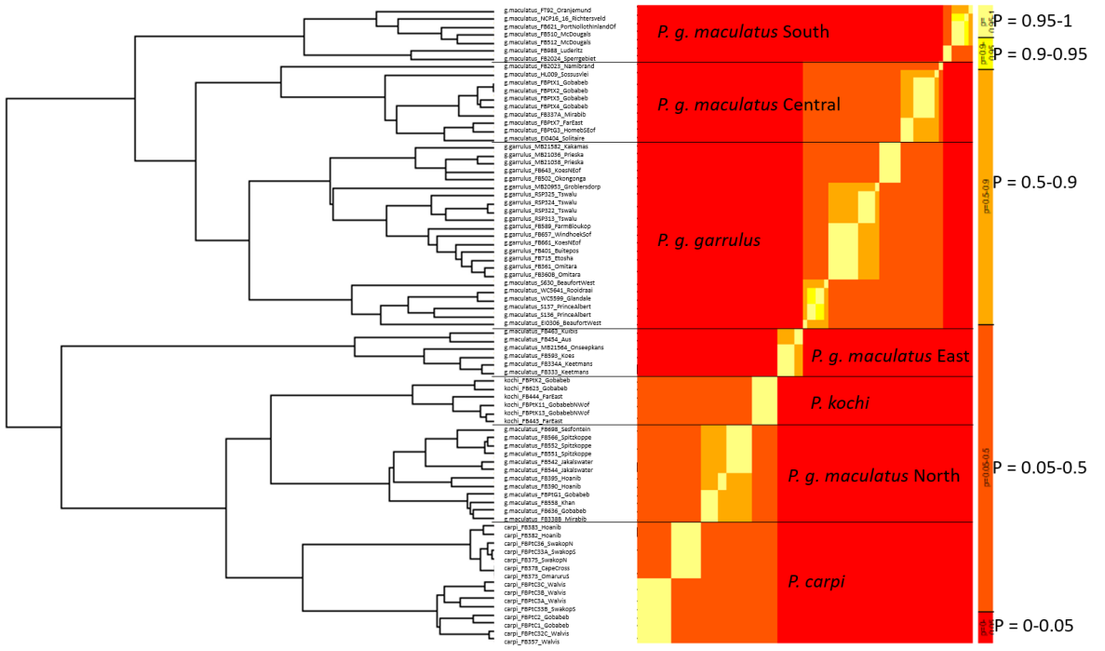

Figure S 11: Output from bGMYC species delimitation on the Beast maximum clade credibility minimum cluster tree from the mitochondrial (*16S* + *ND2*) dataset of *Ptenopus*. The squares in the similarity matrix represent probabilities (coloured scale on the right) for groups of individuals to belong to the same cluster. Sample names relate to the phylogeny in Fig. 4, including sample number and location (see Appendix 1: Table S 1). Lines and labels delineate the “major clades” identified in Chapter 2 for comparison.

Additional morphological results

Table S 3: Scale counts and morphometric measurements used in a principle component analysis of putative *Ptenopus* species included under the accepted species *P. garrulus*.

Field No	Ch. 2 clade	INS	IN/INSBB	RB/RH	INSH/NB	MBSR	IOS
FB333	*P. g. m.* East	1	8.461	1.305	2.316	165	42
FB334B	*P. g. m.* East	1	6.813	1.070	1.029	165	44
FB334A	*P. g. m.* East	1	6.532	1.193	1.739	165	44
FB435	*P. g. m.* East	2	9.850	1.181	0.776	160	50
FB454	*P. g. m.* East	1	7.973	1.361	1.339	160	46
FB462	*P. g. m.* East	1	6.725	0.981	1.244	180	49
FB463	*P. g. m.* East	1	10.983	0.984	0.621	175	46
FB593	*P. g. m.* East	1	7.304	1.316	1.010	161	42
FB323A	*P. g. m.* South	1	8.000	1.013	0.966	160	48
FB321A	*P. g. m.* South	1	5.529	1.132	1.025	157	48
FB328	*P. g. m.* South	2	8.142	0.892	0.545	185	52
FB621	*P. g. m.* South	2	8.846	0.969	0.756	144	41
FB598	*P. g. m.* South	2	10.242	0.949	1.014	141	43
FB510	*P. g. m.* South	4	8.674	1.124	0.618	178	42
FB512	*P. g. m.* South	1	5.203	1.023	0.963	173	44
FB360B	*P. g. garrulus*	1	5.297	1.065	1.165	183	51
FB361	*P. g. garrulus*	1	5.861	1.128	2.024	183	48
FB401	*P. g. garrulus*	1	7.542	1.143	1.890	183	51
FB403	*P. g. garrulus*	1	8.917	1.140	1.091	188	53
FB405	*P. g. garrulus*	3	9.043	1.192	0.333	194	54
FB602	*P. g. garrulus*	2	4.178	1.105	1.188	194	54
FB715	*P. g. garrulus*	1	5.198	0.954	1.058	178	49
FB657	*P. g. garrulus*	2	9.318	0.954	0.671	174	52
FB589	*P. g. garrulus*	1	4.651	1.061	1.350	161	51
FB407	*P. g. garrulus*	2	6.111	1.236	1.273	156	46
FB551	*P. g. m.* North	2	7.973	1.148	0.943	145	38
FB552	*P. g. m.* North	1	5.143	0.974	1.419	156	39
FB390	*P. g. m.* North	1	4.875	1.201	1.650	142	39
FB558	*P. g. m.* North	1	8.826	1.322	2.488	131	32
FB542	*P. g. m.* North	1	8.809	1.136	1.305	135	36
FB566	*P. g. m.* North	1	6.445	1.250	1.824	135	37
FB391	*P. g. m.* North	1	5.591	1.133	2.113	146	40
FBPtG1	*P. g. m.* North	2	8.826	1.322	0.597	144	39
FBPtX1	*P. g. m.* Central	1	4.745	1.309	1.563	108	51
FBPtX2	*P. g. m.* Central	1	3.748	1.246	1.758	131	45
FBPtX3	*P. g. m.* Central	1	4.455	1.242	1.775	134	50
FBPtX7	*P. g. m.* Central	1	4.806	1.268	1.539	136	37
FBPtG3	*P. g. m.* Central	1	3.869	1.411	0.990	144	48
FB2023	*P. g. m.* Central	1	5.313	1.264	1.790	179	46

Table S 4: bGMYC clades *Ptenopus carpi* A and B iris colouration and gular patch data. Samples are arranged from north to south.

Clade	Field No.	Sex	Gular patch	Iris colour	Lat	Lon	Locality	Date
B	FB387	male	yes	silver-brown	-19.353	13.145	near Hoanib River	2019-05-10
B	FB384	male	yes	brown	-19.353	13.145	near Hoanib River	2019-05-11
B	FB398	female	no	brown	-19.354	13.144	near Hoanib River	2019-05-13
B	FB385	male	yes	light red	-19.354	13.145	near Hoanib River	2019-05-11
B	FB386	female	no	brown	-19.354	13.145	near Hoanib River	2019-05-11
B	FB399	male	yes	silver-brown	-19.354	13.145	near Hoanib River	2019-05-13
B	FB400	female	no	brown	-19.355	13.145	near Hoanib River	2019-05-13
B	FB383	male		brown	-19.357	13.133	near Hoanib River	2019-05-10
B	FB553	male	yes	silver-brown	-21.321	13.761	Mile 108, N of	2020-07-12
B	FB556	female	no	brown	-21.322	13.760	Mile 108, N of	2020-07-12
B	FB379	female	no	brown	-21.760	14.001	near Cape Cross, N of Henties Bay	2018-02-26
B	FB378A	male	no	brown	-21.761	14.001	near Cape Cross, N of Henties Bay	2018-02-26
B	FB378B	male	yes	brown	-21.761	14.001	near Cape Cross, N of Henties Bay	2018-02-26
B	FB374	male	yes	brown	-21.770	14.552	N of Omaruru River, 46 km NE of Henties Bay	2018-03-12
B	FB377	female	no	brown	-21.771	14.553	N of Omaruru River, 46 km NE of Henties Bay	2018-03-12
B	FB372	male	yes	silver	-22.078	14.265	N of Omaruru River, Henties Bay	2018-03-11
B	FB371	female	no	silver	-22.158	14.309	Henties Bay, S of Omaruru River	2018-03-11
B	FB373	male	yes	silver-brown	-22.158	14.309	S of Omaruru River, Henties Bay	2018-03-11
B	FB375	male	yes	silver	-22.618	14.538	N of Swakop Swakopmund	2018-02-26
B	FB376	female	no	silver	-22.619	14.538	N of Swakop River, near Swakopmund	2018-02-26
B	FB911	female	no	silver	-22.623	14.531	Swakopmund area	2018-10-08

B	FBPtC35A	female	no	silver	-22.665	14.576	N of Swakop River, near Swakopmund	2018-02-08
B	FBPtC35B	female	no	silver-brown	-22.665	14.576	N of Swakop River, near Swakopmund	2018-02-08
B	FBPtC35C	female	no	brown	-22.665	14.576	N of Swakop River, near Swakopmund	2018-02-08
B	FBPtC36	male	yes	silver	-22.665	14.576	N of Swakop River, near Swakopmund	2018-02-08
A	FB813	juv.			-22.694	14.585	Sakopmund, E of, S of Swakop River	2018-02-08
B	FBPtC33A	female	yes	brown	-22.694	14.585	S of Swakop River near Swakopmund	2018-02-08
A	FBPtC33B	female	yes	red	-22.694	14.585	S of Swakop River near Swakopmund	2018-02-08
AB?	FBPtC34A	male	yes	brown	-22.694	14.585	S of Swakop River near Swakopmund	2018-02-08
A	FB807	male	yes	dark red	-23.000	14.669	Walvis Bay, E of	2018-02-08
A	FB808	male	yes	red	-23.000	14.669	Walvis Bay, E of	2018-02-08
A	FB809	male	yes	light red	-23.000	14.669	Walvis Bay, E of	2018-02-08
A	FB810	male	yes	dark red	-23.000	14.669	Walvis Bay, E of	2018-02-08
A	FB811	male	yes	red	-23.000	14.669	Walvis Bay, E of	2018-02-08
A	FB812	male	yes	light red	-23.000	14.669	Walvis Bay, E of	2018-02-08
A	FBPtC32A	juv.	yes	red	-23.000	14.669	Walvis Bay area	2018-02-08
A	FBPtC32B	female	yes	red	-23.000	14.669	Walvis Bay area	2018-02-08
A	FBPtC32C	female	yes	dark red	-23.000	14.669	Walvis Bay area	2018-02-08
A	FBPtC32D	female	yes	red	-23.000	14.669	Walvis Bay area	2018-02-08
A	FB357	male	yes	dark red	-23.003	14.660	Walvis Bay Airport area	2018-04-18
A	FBPtC3A	female	yes	dark red	-23.010	14.735	Walvis Bay Airport area	2017-09-23
A	FBPtC3B	female	yes	light red	-23.010	14.735	Walvis Bay Airport area	2017-09-23
A	FBPtC3C	male	yes	light red	-23.010	14.735	Walvis Bay Airport area	2017-09-23
A	FBPtC31A	female	yes	red	-23.424	14.935	16 km NW of Gobabeb	2018-02-07
A	FBPtC31B	female	yes	light red	-23.424	14.935	16 km NW of Gobabeb	2018-02-07
A	FBPtC31C	female	yes	red	-23.424	14.935	16 km NW of Gobabeb	2018-02-07
A	FBPtC31D	male	yes	light red	-23.424	14.935	16 km NW of Gobabeb	2018-02-07
A	FBPtC2	male	yes	red	-23.432	14.947	18 km NW of Gobabeb	2017-09-23
A	FBPtC1	female	yes	light red	-23.433	14.946	18 km NW of Gobabeb	2017-09-23

A	FBPtC14	male	yes	light red	-23.434	14.947	Gobabeb, NW of	2017-10-09
A	FBPtC16	male	yes	light red	-23.434	14.946	Gobabeb, NW of	2017-10-09
A	FBPtC12	female	yes	light red	-23.453	14.964	Gobabeb, NW of	2017-10-08
A	FBPtC13	male	yes	red	-23.453	14.964	Gobabeb, NW of	2017-10-08
A	FBPtC11	male	yes	light red	-23.453	14.964	Gobabeb, NW of	2017-10-08
A	FB1100	female	yes	light red	-23.460	14.969	Gobabeb, NW of (Carpi Wash)	2018-02-06
A	FB805	male	yes	red	-23.460	14.969	Gobabeb, NW of (Carpi Wash)	2018-02-06
A	FB1102	male	yes	light red	-23.461	14.967	Gobabeb, NW of (Carpi Wash)	2018-02-06
A	FB1101	female	yes	red	-23.461	14.968	Gobabeb, NW of (Carpi Wash)	2018-02-06
A	FB804	juv	no		-23.466	14.973	Gobabeb, NW of (Carpi Wash)	2018-02-06
A	FBPtC15	female	yes	light red	-23.469	14.974	Gobabeb, NW of	2017-10-08
A	FB802	male	yes	light red	-23.481	14.983	Gobabeb, NW of (Carpi Wash)	2018-02-03
A	FB803	female	yes	light red	-23.481	14.983	Gobabeb, NW of (Carpi Wash)	2018-02-03

Appendix 5: Chapter 4 supplementary data

Table S 5: Additional *Ptenopus* material examined. The type of material (Specimen/Photo/Tissue) collected is indicated by yes/no (Y/N). 'Ref. no.' is either a field number (FB = Francois Becker) or a catalogue number, where available, from the National Museum of Namibia (NMNW: indicated as 'R ####').

Species	Ref. no.	Date	Country	Locality	Lat	Lon	Specimen	Photo	Tissue
P. SP1	FB321A	2018-09-09	Namibia	Lüderitz	-26.613	15.17	N	Y	N
P. SP1	FB323A	2018-09-09	Namibia	Lüderitz	-26.613	15.17	N	Y	N
P. SP1	FB321B	2018-09-12	Namibia	Lüderitz area	-26.617	15.191	N	Y	N
P. SP1	FB323B	2018-09-12	Namibia	Lüderitz area	-26.617	15.191	N	Y	N
P. SP1	FB330	2018-09-14	Namibia	Oranjemund area	-28.552	16.496	N	Y	N
P. SP1	FB329	2018-09-14	Namibia	Oranjemund area	-28.552	16.497	N	Y	N
P. SP1	FB328	2018-09-14	Namibia	Oranjemund area	-28.554	16.498	N	Y	N
P. SP1	FB988	2019-11-26	Namibia	Lüderitz	-26.736	15.095	N	Y	Y
P. SP1	FB509	2020-01-08	South Africa	Port Nolloth area	-29.254	16.868	N	Y	Y
P. SP1	FB510	2020-01-08	South Africa	McDougal's Bay	-29.292	16.882	N	Y	Y
P. SP1	FB512	2020-01-08	South Africa	McDougal's Bay	-29.292	16.882	N	Y	Y
P. SP1	FB513	2020-01-08	South Africa	McDougal's Bay	-29.292	16.882	N	Y	Y
P. SP1	FB514	2020-01-08	South Africa	McDougal's Bay	-29.292	16.882	N	Y	Y
P. SP1	FB516	2020-01-08	South Africa	McDougal's Bay	-29.292	16.882	N	Y	Y
P. SP1	FB614	2020-11-13	South Africa	McDougal's Bay	-29.29	16.883	N	Y	Y
P. SP1	FB630	2020-11-16	South Africa	Port Nolloth, 30 km inland of	-29.309	17.131	N	N	Y
P. SP1	FB621	2020-11-19	South Africa	Port Nolloth, 30 km inland of	-29.305	17.183	N	Y	Y
P. SP1	FB585	2020-11-19	South Africa	Port Nolloth, 30 km inland of	-29.305	17.184	N	Y	Y
P. SP1	FB598	2020-11-19	South Africa	Port Nolloth, 30 km inland of	-29.305	17.184	N	Y	Y

P. SP1	FB645	2020-11-19	South Africa	Port Nolloth, 30 km inland of	-29.305	17.184	N	Y	Y
P. SP1	FB570	2020-11-27	South Africa	Port Nolloth	-29.242	16.866	N	Y	Y
P. SP1	FB647	2020-11-27	South Africa	Port Nolloth	-29.242	16.866	N	Y	Y
P. SP1	FB603	2020-11-27	South Africa	McDougall's Bay	-29.287	16.884	N	Y	Y
P. SP1	FB633	2020-11-27	South Africa	McDougall's Bay	-29.287	16.884	N	Y	Y
P. SP1	FB591	2020-11-28	South Africa	Port Nolloth, inland of	-29.263	16.889	N	Y	Y
P. SP1	R 11390	2022-09-27	Namibia	Grosse Brucht Bay	-26.734	15.104	Y	Y	Y
P. SP1	R 11391	2022-09-27	Namibia	Grosse Brucht Bay	-26.734	15.104	Y	Y	Y
P. SP1	R 11392	2022-09-27	Namibia	Grosse Brucht Bay	-26.734	15.104	Y	Y	N
P. SP1	R 11393	2022-09-27	Namibia	Grosse Brucht Bay	-26.721	15.103	Y	Y	Y
P. SP3	FB332	2018-09-16	Namibia	Campbell farm, Helmeringhausen area	-25.936	16.693	N	Y	N
P. SP3	FB331	2018-09-16	Namibia	Campbell farm, Helmeringhausen area	-25.937	16.693	N	Y	N
P. SP3	FB334A	2018-09-18	Namibia	Keetmanshoop area	-26.55	18.167	N	Y	Y
P. SP3	FB333	2018-09-18	Namibia	Keetmanshoop area	-26.551	18.167	N	Y	Y
P. SP3	FB334B	2018-09-18	Namibia	Keetmanshoop area	-26.551	18.168	N	Y	Y
P. SP3	FB435	2019-11-19	Namibia	Aus area	-26.648	16.215	N	Y	Y
P. SP3	FB456	2019-11-19	Namibia	Aus area	-26.648	16.215	N	Y	Y
P. SP3	FB436	2019-11-19	Namibia	Aus area	-26.648	16.215	N	Y	Y
P. SP3	FB433	2019-11-19	Namibia	Aus area	-26.649	16.216	N	Y	Y
P. SP3	FB453	2019-11-21	Namibia	Aus area	-26.707	16.283	N	Y	Y
P. SP3	FB454	2019-11-21	Namibia	Aus district	-26.709	16.282	N	Y	Y
P. SP3	FB463	2019-11-24	Namibia	farm Kuibis area	-26.621	16.746	N	Y	Y
P. SP3	FB461	2019-11-24	Namibia	farm Kuibis area	-26.622	16.747	N	Y	Y
P. SP3	FB462	2019-11-24	Namibia	farm Kuibis area	-26.622	16.747	N	Y	Y

P. SP3	FB593	2020-10-06	Namibia	Koës	-25.936	19.085	N	Y	Y
P. SP3	FB605	2020-10-06	Namibia	Koës	-25.937	19.085	N	N	Y
P. SP3	FB608	2020-10-06	Namibia	Koës	-25.937	19.089	Y	N	Y
P. SP3	FB604	2020-10-06	Namibia	Koës	-25.937	19.085	Y	N	Y
P. SP3	R 11388	2022-09-27	Namibia	near Giants Playground, Keetmanshoop district	-26.454	18.31	Y	Y	N
P. SP3	R 11389	2022-09-27	Namibia	near Giants Playground, Keetmanshoop district	-26.454	18.31	Y	Y	N
P. SP3	FB2078	2022-09-28	Namibia	Between Aus and Lüderitz	-26.57	15.839	Y	Y	Y
P. SP3	FB2112	2022-09-28	Namibia	Between Aus and Lüderitz	-26.57	15.839	Y	Y	Y
P. SP3	FB2106	2022-09-28	Namibia	Between Aus and Lüderitz	-26.57	15.833	N	Y	Y
P. SP3	FB2053	2022-09-28	Namibia	Between Aus and Lüderitz	-26.605	16.067	Y	Y	Y
P. SP3	FB2088	2022-09-28	Namibia	Between Aus and Lüderitz	-26.578	15.62	N	Y	Y
P. SP3	FB2059	2022-09-28	Namibia	Aus airport	-26.668	16.284	Y	Y	Y
P. SP4	FBPtC18	2017-11-11	Namibia	N of Swakop River, near Swakopmund	-22.662	14.578	N	Y	Y
P. SP4	FBPtC19	2017-11-11	Namibia	N of Swakop River, near Swakopmund	-22.662	14.58	N	Y	Y
P. SP4	FBPtC17	2017-11-11	Namibia	N of Swakop River, near Swakopmund	-22.662	14.58	N	Y	Y
P. SP4	FBPtC20	2017-11-11	Namibia	N of Swakop River, near Swakopmund	-22.665	14.581	N	Y	Y

P. SP4	FBPtC35A	2018-02-08	Namibia	N of Swakop River, near Swakopmund	-22.665	14.576	N	Y	Y
P. SP4	FBPtC35B	2018-02-08	Namibia	N of Swakop River, near Swakopmund	-22.665	14.576	N	Y	Y
P. SP4	FBPtC35C	2018-02-08	Namibia	N of Swakop River, near Swakopmund	-22.665	14.576	N	Y	Y
P. SP4	FBPtC36	2018-02-08	Namibia	N of Swakop River, near Swakopmund	-22.665	14.576	N	Y	Y
P. SP4	FBPtC33A	2018-02-08	Namibia	S of Swakop River near Swakopmund	-22.694	14.585	N	Y	Y
P. SP4	FB379	2018-02-26	Namibia	near Cape Cross, N of Henties Bay	-21.76	14.001	N	Y	Y
P. SP4	FB375	2018-02-26	Namibia	N of Swakop Swakopmund	-22.618	14.538	N	Y	Y
P. SP4	FB376	2018-02-26	Namibia	N of Swakop River, near Swakopmund	-22.619	14.538	N	Y	Y
P. SP4	FB372	2018-03-11	Namibia	N of Omaruru River, Henties Bay	-22.078	14.265	N	Y	Y
P. SP4	FB371	2018-03-11	Namibia	Henties Bay, S of Omaruru River	-22.158	14.309	N	Y	Y
P. SP4	FB373	2018-03-11	Namibia	S of Omaruru River, Henties Bay	-22.158	14.309	N	Y	Y
P. SP4	FB374	2018-03-12	Namibia	N of Omaruru River, 46 km NE of Henties Bay	-21.77	14.552	N	Y	Y
P. SP4	FB377	2018-03-12	Namibia	N of Omaruru River, 46 km NE of Henties Bay	-21.771	14.553	N	Y	Y
P. SP4	FB911	2018-10-08	Namibia	Swakopmund area	-22.623	14.531	N	Y	N
P. SP4	FB919	2019-05-10	Namibia	near Hoanib River	-19.353	13.145	N	Y	N
P. SP4	FB389	2019-05-10	Namibia	near Hoanib River	-19.353	13.146	N	N	Y
P. SP4	FB387	2019-05-10	Namibia	near Hoanib River	-19.353	13.145	N	Y	Y

P. SP4	FB381	2019-05-10	Namibia	near Hoanib River	-19.355	13.146	N	Y	Y
P. SP4	FB382	2019-05-10	Namibia	near Hoanib River	-19.356	13.147	N	Y	Y
P. SP4	FB383	2019-05-10	Namibia	near Hoanib River	-19.357	13.133	N	Y	Y
P. SP4	FB384	2019-05-11	Namibia	near Hoanib River	-19.353	13.145	N	Y	Y
P. SP4	FB385	2019-05-11	Namibia	near Hoanib River	-19.354	13.145	N	Y	Y
P. SP4	FB386	2019-05-11	Namibia	near Hoanib River	-19.354	13.145	N	Y	Y
P. SP4	FB398	2019-05-13	Namibia	near Hoanib River	-19.354	13.144	N	Y	Y
P. SP4	FB399	2019-05-13	Namibia	near Hoanib River	-19.354	13.145	N	Y	Y
P. SP4	FB1011	2019-05-13	Namibia	near Hoanib River	-19.354	13.145	N	Y	N
P. SP4	FB400	2019-05-13	Namibia	near Hoanib River	-19.355	13.145	N	Y	Y
P. SP4	FB553	2020-07-12	Namibia	Mile 108, N of	-21.321	13.761	N	Y	Y
P. SP4	FB556	2020-07-12	Namibia	Mile 108, N of	-21.322	13.76	N	Y	Y
P. SP4	FB555	2020-07-12	Namibia	Mile 108, N of	-21.322	13.759	N	N	Y
P. SP4	FB560	2020-07-12	Namibia	Mile 108, S of	-21.501	13.864	N	N	Y
P. carpi	FBPtC3	2017-09-23	Namibia	Walvis Bay Airport area	-23.01	14.735	N	Y	Y
P. carpi	FBPtC2	2017-09-23	Namibia	18 km NW of Gobabeb	-23.432	14.947	N	Y	Y
P. carpi	FBPtC1	2017-09-23	Namibia	18 km NW of Gobabeb	-23.433	14.946	N	Y	Y
P. carpi	FBPtC10	2017-10-07	Namibia	Gobabeb, NW of (Carpi Wash)	-23.481	14.983	N	Y	Y
P. carpi	FBPtC4	2017-10-07	Namibia	W of Gobabeb	-23.481	14.983	N	Y	Y
P. carpi	FBPtC5	2017-10-07	Namibia	W of Gobabeb	-23.481	14.983	N	Y	Y
P. carpi	FBPtC6	2017-10-07	Namibia	W of Gobabeb	-23.481	14.983	N	Y	Y
P. carpi	FBPtC7	2017-10-07	Namibia	W of Gobabeb	-23.481	14.983	N	Y	Y
P. carpi	FBPtC8	2017-10-07	Namibia	W of Gobabeb	-23.481	14.983	N	Y	Y
P. carpi	FBPtC9	2017-10-07	Namibia	W of Gobabeb	-23.481	14.983	N	Y	Y
P. carpi	FBPtC12	2017-10-08	Namibia	Gobabeb, NW of	-23.453	14.964	N	Y	Y
P. carpi	FBPtC13	2017-10-08	Namibia	Gobabeb, NW of	-23.453	14.964	N	Y	N

P. carpi	FBPtC11	2017-10-08	Namibia	Gobabeb, NW of	-23.453	14.964	N	Y	Y
P. carpi	FBPtC15	2017-10-08	Namibia	Gobabeb, NW of	-23.469	14.974	N	Y	Y
P. carpi	FBPtC14	2017-10-09	Namibia	Gobabeb, NW of	-23.434	14.947	N	Y	N
P. carpi	FBPtC16	2017-10-09	Namibia	Gobabeb, NW of	-23.434	14.946	N	Y	N
P. carpi	FB445	2017-11-07	Namibia	Carpi Wash, 10km NW of Gobabeb	-23.481	14.983	N	N	Y
P. carpi	FBPtC25	2017-11-13	Namibia	Swakopmund, E of	-22.687	14.588	N	Y	Y
P. carpi	FBPtC21	2017-11-13	Namibia	Swakopmund, E of	-22.689	14.587	N	Y	N
P. carpi	FBPtC22	2017-11-13	Namibia	Swakopmund, E of	-22.689	14.587	N	Y	N
P. carpi	FB1004	2017-11-13	Namibia	Swakopmund, E of	-22.689	14.588	N	Y	Y
P. carpi	FBPtC23	2017-11-13	Namibia	Swakopmund, E of	-22.69	14.587	N	Y	Y
P. carpi	FB802	2018-02-03	Namibia	Gobabeb, NW of (Carpi Wash)	-23.481	14.983	N	Y	N
P. carpi	FB803	2018-02-03	Namibia	Gobabeb, NW of (Carpi Wash)	-23.481	14.983	N	Y	N
P. carpi	FB805	2018-02-06	Namibia	Gobabeb, NW of (Carpi Wash)	-23.46	14.969	N	Y	N
P. carpi	FB1100	2018-02-06	Namibia	Gobabeb, NW of (Carpi Wash)	-23.46	14.969	N	Y	Y
P. carpi	FB1102	2018-02-06	Namibia	Gobabeb, NW of (Carpi Wash)	-23.461	14.967	N	Y	Y
P. carpi	FB1101	2018-02-06	Namibia	Gobabeb, NW of (Carpi Wash)	-23.461	14.968	N	Y	Y
P. carpi	FB804	2018-02-06	Namibia	Gobabeb, NW of (Carpi Wash)	-23.466	14.973	N	Y	N
P. carpi	FBPtC31A	2018-02-07	Namibia	16 km NW of Gobabeb	-23.424	14.935	N	Y	Y
P. carpi	FBPtC31B	2018-02-07	Namibia	16 km NW of Gobabeb	-23.424	14.935	N	Y	Y
P. carpi	FBPtC31C	2018-02-07	Namibia	16 km NW of Gobabeb	-23.424	14.935	N	Y	Y

P. carpi	FBPtC31D	2018-02-07	Namibia	16 km NW of Gobabeb	-23.424	14.935	N	Y	Y
P. carpi	FBPtC33B	2018-02-08	Namibia	S of Swakop River near Swakopmund	-22.694	14.585	N	Y	Y
P. carpi	FBPtC34A	2018-02-08	Namibia	S of Swakop River near Swakopmund	-22.694	14.585	N	Y	Y
P. carpi	FBPtC34B	2018-02-08	Namibia	S of Swakop River near Swakopmund	-22.694	14.585	N	Y	Y
P. carpi	FB813	2018-02-08	Namibia	Sakopmund, E of, S of Swakop River	-22.694	14.585	N	Y	N
P. carpi	FBPtC32A	2018-02-08	Namibia	Walvis Bay area	-23	14.669	N	N	Y
P. carpi	FBPtC32B	2018-02-08	Namibia	Walvis Bay area	-23	14.669	N	Y	Y
P. carpi	FBPtC32C	2018-02-08	Namibia	Walvis Bay area	-23	14.669	N	Y	Y
P. carpi	FBPtC32D	2018-02-08	Namibia	Walvis Bay area	-23	14.669	N	Y	Y
P. carpi	FB807	2018-02-08	Namibia	Walvis Bay, E of	-23	14.669	N	Y	N
P. carpi	FB808	2018-02-08	Namibia	Walvis Bay, E of	-23	14.669	N	Y	N
P. carpi	FB809	2018-02-08	Namibia	Walvis Bay, E of	-23	14.669	N	Y	N
P. carpi	FB810	2018-02-08	Namibia	Walvis Bay, E of	-23	14.669	N	Y	N
P. carpi	FB811	2018-02-08	Namibia	Walvis Bay, E of	-23	14.669	N	Y	N
P. carpi	FB357	2018-04-18	Namibia	Walvis Bay Airport area	-23.003	14.66	N	Y	Y
P. carpi	FB820	2018-04-18	Namibia	Walvis Bay Airport area	-23.003	14.66	N	Y	N
P. SP2	FBPtX1	2017-09-07	Namibia	Gobabeb	-23.567	15.038	N	Y	Y
P. SP2	FBPtX2	2017-09-10	Namibia	Gobabeb	-23.569	15.037	N	Y	Y
P. SP2	FBPtX3	2017-09-12	Namibia	Gobabeb	-23.567	15.038	N	Y	Y
P. SP2	FBPtX4	2017-09-24	Namibia	Gobabeb	-23.567	15.037	N	N	Y
P. SP2	FBPtX7	2017-09-26	Namibia	Far East dunes, NW of Solitaire	-23.793	15.78	N	Y	Y
P. SP2	FB755	2017-09-27	Namibia	SE of Homeb	-23.698	15.348	N	Y	N
P. SP2	FBPtG4	2017-09-27	Namibia	SE of Homeb	-23.698	15.348	N	N	Y

P. SP2	FBPtG3	2017-09-27	Namibia	SE of Gobabeb	-23.698	15.348	N	Y	Y
P. SP2	FBPtX5	2017-10-03	Namibia	Gobabeb	-23.569	15.038	N	Y	Y
P. SP2	FBPtG8	2017-11-03	Namibia	Mirabib	-23.469	15.363	N	Y	Y
P. SP2	FBPtX10	2017-11-07	Namibia	Mirabib	-23.45	15.356	N	Y	N
P. SP2	FBPtX9	2017-11-07	Namibia	Mirabib	-23.451	15.356	N	Y	Y
P. SP2	FBPtG10	2017-11-07	Namibia	Mirabib	-23.451	15.356	N	Y	Y
P. SP2	FBPtX8	2017-11-07	Namibia	Mirabib	-23.456	15.383	N	Y	Y
P. SP2	FB337B	2018-12-15	Namibia	Mirabib	-23.468	15.359	N	Y	Y
P. SP2	FB337C	2018-12-15	Namibia	Mirabib	-23.468	15.359	N	Y	Y
P. SP2	FB337A	2018-12-15	Namibia	Mirabib	-23.471	15.363	N	Y	Y
P. SP2	HL009	2020-09-05	Namibia	Kulala Deser Lodge	-24.622	15.721	N	N	Y
P. SP2	FB2021	2021-09-17	Namibia	Gobabeb, across Kuiseb from	-23.57	15.039	Y	N	Y
P. SP2	FB2022	2021-09-17	Namibia	Gobabeb, across Kuiseb from	-23.57	15.039	Y	N	Y
P. SP2	FB2023	2021-09-20	Namibia	Keerwerder, NamibRand	-24.95	16.04	Y	Y	Y
P. SP2	FB2080	2022-09-05	Namibia	C14, NW of Gobabeb	-23.317	15.57	Y	N	Y
P. SP2	R 11394	2022-09-06	Namibia	Gobabeb, south of the Kuiseb River	-23.571	15.036	Y	Y	N
P. SP2	R 11395	2022-09-06	Namibia	Gobabeb, south of the Kuiseb River	-23.571	15.036	Y	Y	N
P. SP2	R 11396	2022-09-06	Namibia	Gobabeb, south of the Kuiseb River	-23.571	15.036	Y	Y	N
P. SP2	FB2050	2022-09-07	Namibia	C14 & C26 T-junction, E of Kuiseb River	-23.353	15.855	Y	N	Y
P. SP2	FB2003	2022-09-07	Namibia	Keerwerder, Namibrand	-24.982	15.934	Y	Y	Y

P. SP2	FB2017	2022-09-07	Namibia	Keerwerder, Namibrand	-24.954	16.035	Y	Y	Y
P. SP2	FB2073	2022-09-07	Namibia	Keerwerder, Namibrand	-24.977	15.93	N	Y	Y
P. SP2	FB2077	2022-09-07	Namibia	Keerwerder, Namibrand	-24.977	15.93	N	Y	Y
P. SP2	FB2085	2022-09-07	Namibia	Keerwerder, Namibrand	-24.977	15.93	N	Y	Y
P. SP2	FB2120	2022-09-07	Namibia	Keerwerder, Namibrand	-24.977	15.93	Y	N	Y
P. garrulus	FB361	2019-09-18	Namibia	Omitara area	-22.271	18.015	N	Y	Y
P. garrulus	FB360B	2019-09-18	Namibia	Omitara area	-22.279	18.014	N	Y	Y
P. garrulus	FB414B	2019-09-18	Namibia	Omitara	-22.28	18.013	N	N	Y
P. garrulus	FB401	2019-10-02	Namibia	40km SW of Buitepos	-22.575	19.735	N	Y	Y
P. garrulus	FB402	2019-10-02	Namibia	40km SW of Buitepos	-22.575	19.735	N	Y	Y
P. garrulus	FB403	2019-10-02	Namibia	40km SW of Buitepos	-22.575	19.735	N	Y	Y
P. garrulus	FB416	2019-10-02	Namibia	40km SW of Buitepos	-22.576	19.735	Y	N	Y
P. garrulus	FB405	2019-10-03	Namibia	Kalahari Bush Breaks farm, near Buitepos	-22.338	19.753	N	N	Y
P. garrulus	FB408B	2019-10-03	Namibia	Kalahari Bush Breaks farm, near Buitepos	-22.338	19.753	N	Y	Y
P. garrulus	FB407	2019-10-03	Namibia	Kalahari Bush Breaks farm, near Buitepos	-22.338	19.753	N	Y	Y
P. garrulus	FB415	2019-10-03	Namibia	Kalahari Bush Breaks	-22.338	19.753	Y	N	Y
P. garrulus	FB502	2020-01-24	Namibia	Okongonga Guest Farm, Stampriet area	-24.453	18.763	N	Y	Y
P. garrulus	FB622	2020-10-07	Namibia	NE of Koës	-25.711	19.306	Y	Y	Y

P. garrulus	FB643	2020-10-07	Namibia	NE of Koës	-25.711	19.306	Y	Y	Y
P. garrulus	FB661	2020-10-07	Namibia	NE of Koës	-25.711	19.306	Y	Y	Y
P. garrulus	FB642	2020-10-07	Namibia	NE of Koës	-25.711	19.306	Y	N	N
P. garrulus	FB589	2020-10-08	Namibia	Farm Bloukop, Namibia	-25.095	19.85	Y	Y	Y
P. garrulus	FB602	2020-10-08	Namibia	Farm Bloukop, Namibia	-25.095	19.85	N	Y	Y
P. garrulus	FB594	2020-10-08	Namibia	Farm Bloukop, Namibia	-25.096	19.849	Y	Y	Y
P. garrulus	FB640	2020-10-08	Namibia	Farm Bloukop, Namibia	-25.097	19.848	N	Y	Y
P. garrulus	FB635	2020-10-08	Namibia	Farm Bloukop, Namibia	-25.097	19.849	N	Y	Y
P. garrulus	FB657	2020-11-03	Namibia	Windhoek, 40 km south of	-22.869	17.156	N	Y	Y
P. garrulus	FB731	2020-11-03	Namibia	Windhoek, 40 km south of	-22.869	17.155	Y	N	Y
P. garrulus	FB637	2020-11-03	Namibia	Windhoek, 40 km south of	-22.869	17.155	Y	Y	Y
P. garrulus	FB2020	2020-11-03	Namibia	Windhoek, 40 km south of	-22.869	17.155	Y	N	Y
P. garrulus	FB668	2021-10-20	Namibia	Okondeka, Etosha National Park	-18.998	15.866	N	Y	Y
P. garrulus	FB995	2021-10-20	Namibia	Okondeka, Etosha National Park	-18.998	15.866	Y	Y	Y
P. garrulus	FB728	2021-10-20	Namibia	Okondeka, Etosha National Park	-18.998	15.866	Y	Y	Y
P. garrulus	FB724	2021-10-20	Namibia	Okondeka, Etosha National Park	-18.998	15.866	N	Y	Y
P. garrulus	FB715	2021-10-20	Namibia	Okondeka, Etosha National Park	-18.998	15.866	Y	Y	Y

P. garrulus	FB726	2021-10-21	Namibia	Okondeka, Etosha National Park	-18.998	15.866	N	Y	Y
P. garrulus	FB701	2021-10-21	Namibia	Okondeka, Etosha National Park	-18.998	15.867	N	N	Y
P. kochi	FB732	2017-07-08	Namibia	Gobabeb	-23.562	15.04	N	Y	N
P. kochi	FB733	2017-07-10	Namibia	Gobabeb	-23.562	15.04	N	Y	N
P. kochi	FB739	2017-08-19	Namibia	Gobabeb	-23.562	15.04	N	Y	N
P. kochi	FBPtK3	2017-09-26	Namibia	Homeb, SE of (Far East Dunes)	-23.795	15.783	N	Y	Y
P. kochi	FBPtK1	2017-10-03	Namibia	Gobabeb	-23.578	15.042	N	Y	Y
P. kochi	FBPtK2	2017-10-06	Namibia	Gobabeb	-23.56	15.037	N	Y	Y
P. kochi	FBPtK9	2017-10-15	Namibia	Gobabeb, NW of	-23.361	14.818	N	Y	Y
P. kochi	FBPtK10	2017-10-15	Namibia	Gobabeb, NW of	-23.361	14.818	N	Y	Y
P. kochi	FBPtK8	2017-10-15	Namibia	Gobabeb, NW of	-23.361	14.819	N	Y	Y
P. kochi	FBPtK13	2017-10-16	Namibia	Gobabeb, NW of	-23.359	14.817	N	Y	Y
P. kochi	FB773	2017-10-16	Namibia	Gobabeb, NW of	-23.36	14.818	N	Y	N
P. kochi	FBPtK11	2017-10-16	Namibia	Gobabeb, NW of	-23.36	14.818	N	Y	Y
P. kochi	FB729	2017-11-02	Namibia	Gobabeb	-23.562	15.041	N	N	Y
P. kochi	FB444	2018-03-15	Namibia	Far East dunes	-23.681	15.647	N	Y	Y
P. kochi	FB443	2018-03-15	Namibia	Far East dunes	-23.682	15.647	N	Y	Y
P. kochi	FB623	2020-09-14	Namibia	Gobabeb	-23.561	15.038	N	N	Y
P. kochi	FB660	2020-09-15	Namibia	Gobabeb	-23.562	15.039	N	N	Y
P. kochi	FB634	2020-09-17	Namibia	Gobabeb	-23.561	15.04	N	Y	Y
P. kochi	FB631	2020-09-17	Namibia	Gobabeb	-23.561	15.04	N	Y	Y
P. maculatus	FB1014	2017-06-14	Namibia	Gobabeb	-23.562	15.04	N	Y	N
P. maculatus	FB737	2017-08-01	Namibia	Gobabeb	-23.562	15.039	N	Y	N
P. maculatus	FB740	2017-08-20	Namibia	Gobabeb	-23.561	15.039	N	Y	N
P. maculatus	FB1015	2017-08-21	Namibia	Gobabeb	-23.562	15.04	N	Y	N
P. maculatus	FB741	2017-08-21	Namibia	Gobabeb	-23.562	15.04	N	Y	N
P. maculatus	FB742	2017-08-22	Namibia	Gobabeb	-23.562	15.04	N	Y	N

P. maculatus	FBPtG1	2017-09-09	Namibia	Walvis Bay, 48 km SE of	-23.184	14.927	N	Y	Y
P. maculatus	FBPtG2	2017-10-06	Namibia	Gobabeb	-23.56	15.037	N	Y	N
P. maculatus	FBPtG5	2017-10-06	Namibia	Gobabeb	-23.569	15.038	N	Y	Y
P. maculatus	FBPtG6	2017-10-07	Namibia	Gobabeb	-23.561	15.039	N	Y	Y
P. maculatus	FB760	2017-10-13	Namibia	Gobabeb	-23.562	15.04	N	Y	N
P. maculatus	FBPtG9	2017-11-04	Namibia	Gobabeb	-23.562	15.04	N	Y	Y
P. maculatus	FBPtG11	2017-11-07	Namibia	Mirabib	-23.451	15.356	N	Y	Y
P. maculatus	FB871	2018-08-11	Namibia	Gobabeb	-23.562	15.04	N	Y	N
P. maculatus	FB881	2018-08-13	Namibia	Gobabeb	-23.561	15.04	N	Y	N
P. maculatus	FB338A	2018-12-15	Namibia	Mirabib	-23.468	15.359	N	Y	Y
P. maculatus	FB338B	2018-12-15	Namibia	Mirabib	-23.468	15.359	N	Y	Y
P. maculatus	FB338C	2018-12-15	Namibia	Mirabib	-23.468	15.359	N	Y	Y
P. maculatus	FB392	2019-05-12	Namibia	Hoanib River	-19.362	13.15	N	Y	Y
P. maculatus	FB394	2019-05-12	Namibia	Hoanib River	-19.362	13.15	N	Y	Y
P. maculatus	FB393	2019-05-12	Namibia	Hoanib River	-19.362	13.15	N	Y	Y
P. maculatus	FB390	2019-05-12	Namibia	Hoanib River	-19.362	13.15	N	Y	Y
P. maculatus	FB391	2019-05-12	Namibia	Hoanib River	-19.362	13.15	N	Y	Y
P. maculatus	FB395	2019-05-12	Namibia	Hoanib River	-19.363	13.15	N	Y	Y
P. maculatus	FB550	2020-06-08	Namibia	farm Jakalswater, Karibib District	-22.588	15.37	N	Y	Y
P. maculatus	FB557	2020-06-08	Namibia	farm Jakalswater, Karibib District	-22.592	15.37	N	Y	Y
P. maculatus	FB542	2020-06-08	Namibia	farm Jakalswater, Karibib District	-22.595	15.375	N	Y	Y
P. maculatus	FB544	2020-06-08	Namibia	farm Jakalswater, Karibib District	-22.595	15.375	N	Y	Y
P. maculatus	FB548	2020-06-08	Namibia	farm Jakalswater, Karibib District	-22.595	15.375	N	Y	Y

P. maculatus	FB558	2020-06-09	Namibia	Khan River, Karibib Distrit	-22.423	15.122	N	Y	Y
P. maculatus	FB551	2020-06-10	Namibia	Spitzkoppe	-21.838	15.195	N	Y	Y
P. maculatus	FB566	2020-06-10	Namibia	Spitzkoppe	-21.838	15.195	N	Y	Y
P. maculatus	FB552	2020-06-10	Namibia	Spitzkoppe	-21.838	15.195	N	Y	Y
P. maculatus	FB565	2020-06-10	Namibia	Spitzkoppe	-21.839	15.195	N	Y	Y
P. maculatus	FB540	2020-06-11	Namibia	Spitzkoppe	-21.839	15.194	N	N	Y
P. maculatus	FB636	2020-09-15	Namibia	Gobabeb	-23.561	15.039	Y	N	Y
P. maculatus	FB699	2021-04-21	Namibia	Sesfontein Lodge	-19.119	13.621	N	N	Y
P. maculatus	FB2035	2021-04-21	Namibia	Sesfontein Lodge	-19.119	13.621	Y	Y	Y
P. maculatus	FB698	2021-04-21	Namibia	Sesfontein Lodge	-19.119	13.621	N	N	Y
P. maculatus	FB2012	2021-09-18	Namibia	Gobabeb	-23.561	15.039	Y	N	Y

Appendix 6: Chapter 4 supplementary results

Table S 6: Summary of all call variables measured for *Ptenopus* species, during chorus time. The mean, minimum, and maximum is indicated for each variable.

Variable	*P. carpi*	*P.* SP4	*P. garrulus*	*P. kochi*	*P. maculatus*	*P.* SP1	*P.* SP2	*P.* SP3
n calls	18	30	187	151	252	40	89	72
n individuals	3	6	21	17	28	4	9	8
n notes								
mean	9.73	9.88	5.37	13.43	6.03	4.88	4.53	4.59
min	9.00	8.00	4.10	10.60	4.00	3.80	2.70	3.92
max	11.20	13.00	6.25	19.33	7.30	6.20	6.60	5.10
note repetition rate (s^{-1})								
mean	2.25	2.60	5.28	7.90	4.93	2.86	2.40	2.45
min	1.68	1.25	3.76	5.98	2.58	2.65	1.57	1.99
max	3.01	4.32	6.44	10.33	7.17	7.00	2.81	2.96
note duration median (ms)								
mean	25.89	28.29	21.71	27.48	50.37	28.48	48.13	92.05
min	22.28	20.86	15.84	18.45	26.25	24.18	31.17	60.86
max	28.55	40.27	30.71	40.23	70.61	33.85	56.96	154.40
note 1 duration deviance (% of median)								
mean	12.80	8.69	13.47	27.42	137.26	9.13	15.97	30.08
min	5.45	0.50	-2.99	6.23	105.71	-4.20	2.77	9.66
max	24.82	16.23	27.46	44.90	173.65	15.71	27.92	47.17
inter-note interval median (ms)								
mean	560.02	386.83	167.80	98.32	146.59	308.02	393.95	315.97
min	323.23	204.48	113.97	74.96	88.00	274.38	237.23	268.99
max	681.58	661.04	234.03	130.41	302.59	341.04	586.94	368.03
inter-note interval range (% of median)								

mean	84.00	101.90	25.25	29.98	20.18	52.55	12.11	23.32
min	61.72	26.27	14.11	13.90	9.51	36.28	10.08	11.63
max	105.14	163.39	34.63	51.54	42.40	66.42	15.97	42.33
median call density								
mean	0.06	0.08	0.13	0.28	0.36	0.09	0.13	0.29
min	0.05	0.06	0.09	0.20	0.17	0.08	0.09	0.18
max	0.08	0.11	0.18	0.34	0.50	0.10	0.17	0.48
call duration (s)								
mean	3.57	3.91	0.86	1.62	1.09	1.39	1.50	1.57
min	3.32	2.49	0.57	1.18	0.84	1.09	1.12	1.29
max	3.80	7.21	1.23	2.43	1.40	1.82	2.04	1.86
(upper) dominant frequency (kHz)								
mean	3.49	4.27	4.12	3.31	3.38	4.27	3.82	3.51
min	3.30	3.80	3.91	3.07	2.99	4.08	3.46	3.26
max	3.82	4.78	4.56	3.47	3.79	4.47	4.13	3.80
bandwidth upper (Hz)								
mean	4653	6925	8166	4874	4764	7721	4653	6886
min	3794	4755	4311	3299	3501	5500	3864	4276
max	5426	12016	13183	10556	11757	12370	5194	12915
bandwidth lower (Hz)								
mean	194	296	576	689	911	500	899	531
min	108	112	416	296	231	327	203	129
max	258	495	1193	1115	1335	715	1301	1034