Out of the blue: a novel, trans-Atlantic clade of geckos (Gekkota, Squamata)

TONY GAMBLE, AARON M. BAUER, ELI GREENBAUM & TODD R. JACKMAN

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Phylogenetic relationships among gekkotan lizards were estimated from five nuclear protein-coding genes in separate and combined analyses using maximum parsimony, maximum likelihood and Bayesian analyses. All analyses recovered a monophyletic trans-Atlantic gecko clade (Phyllodactylidae) consisting of the genera *Asaccus*, *Haemodracon*, *Homonota*, *Phyllodactylus*, *Phyllopezus*, *Ptyodactylus*, *Tarentola* and *Thecadactylus*. No other phylogenetic or taxonomic hypotheses have proposed linking these genera, which have been consistently grouped with other taxa outside of the clade. In this paper, we determine the relationships of this new clade to other major gekkotan groups, evaluate previous phylogenetic hypotheses regarding constituent members of this novel clade, and critically examine the use of historically important morphological characters in gekkotan systematics as they relate to this novel clade, specifically — phalangeal formulae, hyoid morphology and external structure of the toe-pads.

Corresponding author: *Tony Gamble*, *Conservation Biology Graduate Program*, *Bell Museum of Natural History*, *University of Minnesota*, *St. Paul*, *MN 55108*, *USA. E-mail: gambl007@umn.edu*
Aaron M. Bauer, *Department of Biology*, *Villanova University*, *Villanova*, *PA*, *USA. E-mail: aaron.bauer@villanova.edu*
Eli Greenbaum, *Department of Biology*, *Villanova University*, *Villanova*, *PA*, *USA. E-mail: eli.greenbaum@villanova.edu*
Todd R. Jackman, *Department of Biology*, *Villanova University*, *800 Lancaster Avenue*, *Villanova*, *PA 19085*, *USA. E-mail: todd.jackman@villanova.edu*

Introduction

Cryptic species are distinct evolutionary lineages that are, superficially, undiagnosable using morphological characters (Bickford *et al*. 2006; Egge & Simons 2006). Numerous cryptic species have been described or identified based on molecular data (Highton *et al*. 1989; Burbrink 2002; Leaché & Reeder 2002; Egge & Simons 2006; Bergmann & Russell 2007). There seems to be little controversy that molecular data may be a better tool, in some cases, than morphology in identifying taxa at the species level. The same could be said for identifying higher-level taxa as well. Recent examples of unexpected, higher-level taxonomic groups, discovered using DNA data, include a clade of morphologically diverse African mammals, the Afrotheria (Stanhope *et al*. 1998); a clade of moulting metazoans uniting, arthropods with nematodes, the Ecdysozoa (Aguinaldo *et al*. 1997); and a clade of venomous lizards and snakes, the Toxicofera (Fry *et al*. 2005; Vidal & Hedges 2005). What common features might account for the failure to identify these clades prior to their discovery using genetic data? First, they involve taxonomically rich and geographically widespread groups for which it has been difficult to sample representative taxa. Thorough taxon sampling can have a profound impact on phylogenetic reconstruction (Hillis 1996, 1998; Graybeal 1998; Hedtke *et al*. 2006). Second, phylogentic signal in the morphological data sets used thus far appear to have been masked by convergence or parallelism, often because homoplastic characters are more evident or because historically, certain characters have been accorded overarching importance in the taxonomy of certain groups. This has certainly been the case with gekkotan lizards, where a small number of morphological characters, particularly external digital morphology, have long been the primary basis for the recognition and erection of genera.

Geckos (Squamata: Gekkota) are a species-rich and geographically widespread group of lizards. Previous phylogenetic and taxonomic treatments have offered hypotheses regarding higher-level relationships (e.g. Underwood 1954; Kluge 1967, 1987, 1995; Han *et al*. 2004; Feng *et al*. 2007; Gamble *et al*. 2008a) and species-level relationships in certain groups (e.g. Macey *et al*. 1999; Carranza *et al*. 2002; Lamb & Bauer 2002, 2006; Austin *et al*. 2004; Melville *et al*. 2004; Bauer & Lamb 2005; Carranza & Arnold 2006;

Greenbaum *et al*. 2007a,b; Oliver *et al*. 2007; Gamble *et al*. 2008b; Jackman *et al*. 2008). Numerous attempts have been made to resolve the relationships among genera within families with varying levels of resolution (summarized in Russell & Bauer 2002). Robust phylogenies exist for relationships among genera within Eublepharidae (Grismer 1988; Ota *et al*. 1999); Carphodactylidae (Bauer 1990a); Pygopodidae (Jennings *et al*. 2003); and Sphaerodactylidae (Gamble *et al*. 2008a). The remaining major Gekkotan clades, Diplodactylidae and Gekkonidae, have been the subject of attempts at generic-level phylogenies but none has achieved dense generic sampling or utilized enough data to consistently recover intergeneric groups (Russell 1976; Joger 1985; Kluge 1987; Bauer 1990b; Kluge & Nussbaum 1995; Donnellan *et al*. 1999; Han *et al*. 2004; Oliver *et al*. 2007). One exception has been the grouping of five genera in the southern African *Pachydactylus* group with the North African genus *Tarentola*. This grouping is based on a single synapomorphy, an additional phalangeal bone in the first digits of both manus and pes. This unique hyperphalangeal formula has been observed in the following Gekkotan genera: *Pachydactylus*, *Chondrodactylus*, *Colopus*, *Elasmodactylus*, *Rhoptropus* (the *Pachydactylus* group), and *Tarentola* (including *Geckonia sensu* Carranza *et al*. 2002) (Russell 1972). Hyperphalangy has also been observed in the padless Gekkotan species *Cnemaspis chanthaburiensis*, which possesses additional phalangeal bones in digit two of manus and pes, and digit five of the manus (Bauer & Das 1998). The apparent rarity of hyperphalangy in geckos has led numerous authors to assert the monophyly of the *Pachydactylus* group + *Tarentola* as one of the only well-supported, generic level relationships amongst the otherwise phylogenetically intractable Gekkonidae (Russell 1972; Haacke 1976; Kluge 1987; Bauer 1990b; Kluge & Nussbaum 1995; Lamb & Bauer 2002, 2006; Bauer & Lamb 2005).

As part of a broader study of relationships across all gekkotan lizards, we re-evaluated the purported affinities of the largely North African/Mediterranean *Tarentola* to the southern African *Pachydactylus* group. Our findings not only suggest that these two groups are not closely allied, but also led to the identification of a novel higher order group within Gekkota that further emphasizes the inappropriateness of single morphological characters as evidence of shared ancestry. Utilizing multiple nuclear markers and robust generic sampling, we provide the first phylogenetic hypothesis of this novel clade of geckos. Our objectives with this paper are: (i) to generate a phylogeny of this new clade and determine its relationships to other major gekkotan clades; (ii) evaluate previous phylogenetic hypotheses regarding constituent members of this novel clade; and (iii) critically examine the use of historically important morphological characters in gekkotan systematics as they relate to this novel clade, specifically — phalangeal formulae, hyoid morphology and external structure of the toe-pads. Phalangeal formulae, as discussed above, have been used to unite the African *Pachydactylus* group + *Tarentola*. The hyoid apparatus, a group of thin bones or cartilages that provides support to the tongue, has provided important characters for use in higher-level gecko systematic research (Kluge 1967, 1983). The ancestral lizard hyoid consists of three bony or cartilaginous arches that spread posteriorly from the central basihyal element (Romer 1956). The absence of the second ceratobranchial arch is considered the derived condition within geckos and was the sole synapomorphy defining the clade Gekkonini, which consists of the following gekkotan genera: *Agamura*, *Ailuronyx*, *Alsophylax*, *Aristelliger*, *Bogertia*, *Bunopus*, *Calodactylodes*, *Carinatogecko*, *Cnemaspis*, *Crossobamon*, *Cyrtopodion*, *Geckolepis*, *Gehyra*, *Gekko*, *Gymnodactylus*, *Hemidactylus*, *Hemiphyllodactylus*, *Heteronotia*, *Homopholis*, *Lepidodactylus*, *Luperosaurus*, *Lygodactylus*, *Perochirus*, *Phyllopezus*, *Pseudogekko*, *Ptychozoon*, *Stenodactylus*, *Teratolepis*, *Thecadactylus*, *Tropiocolotes*, *Urocotyledon* and *Uroplatus* (Kluge 1983, 1987). External digital morphology has historically been the sole or primary basis for delimiting genera and assigning them to higher-level groupings and includes the presence or absence of digital lamellae as well as the shape and pattern of lamellae (e.g. Fitzinger 1843; Boulenger 1885; Loveridge 1947; Vanzolini 1968).

Materials and methods

We sampled representative species and genera from the Gekkonidae, *sensu* Gamble *et al*. (2008a) and exemplars from each of the remaining gekkotan families, for example, Carphodactylidae, Diplodactylidae, Eublepharidae, Pygopodidae and Sphaerodactylidae. The skink, *Tiliqua rugosa*, and amphisbaenian, *Rhineura floridana*, were used as outgroups. The basal position of geckos with relation to other squamates (Townsend *et al*. 2004; Vidal & Hedges 2005) means that any non-gekkotan squamates are equally appropriate outgroups. Locality data, museum catalogue numbers or field numbers, and GenBank accession numbers for sampled taxa are listed in Table 1.

We extracted genomic DNA from muscle, liver or tail clips using the DNeasy Blood & Tissue kit (Qiagen, Venlo, the Netherlands). We used PCR to amplify portions of five nuclear protein-coding genes: recombination activating gene 1 (RAG1); recombination activating gene 2 (RAG2); oocyte maturation factor MOS (c-mos); acetylcholinergic receptor M4 (ACM4 or CHRM4); and phosducin (PDC). All included sequences were protein-coding only and did not include introns or promoters.

Primers used are listed in Table 2. We used the following PCR profile for RAG2, ACM4 and c-mos: an initial 5 min denaturation at 94 °C followed by 32 cycles of denaturation (30 s at 94 °C), annealing (45 s at 52 °C) and extension (1 min at 72 °C), followed by a final extension of 5 min at 72 °C.

Table 1 Details of material examined. Family names abbreviated: E, Eublepharidae, D, Diplodactylidae, C, Carphodactylidae, Py, Pygopodidae, S, Sphaerodactylidae, Ph, Phyllodactylidae, and G, Gekkonidae. Museum abbreviations follow Leviton *et al*. (1985) except as follows: AMB, Aaron M. Bauer; ENS, Eric N. Smith; FG/MV, Frank Glaw and Miguel Vences; JB, Jon Boone; JS, Jay Sommers; JV, Jens Vindum; LJAMM, Luciano J. Avila and Mariana Morando; LLG, L. Lee Grismer; MF, Mike Forstner; TG, Tony Gamble.

				Genbank accession numbers				
Family	Species	Specimen ID	Locality	RAG1	RAG2	c-mos	ACM4	PDC
E	*Eublepharis macularius*	TG 00081	Pakistan	—	EF534942	EF534900	EF534857	—
E	*Eublepharis macularius*	JS2	Pakistan	EF534776	—	—	—	EF534816
E	*Coleonyx variegatus*	CAS 205334	California, USA	EF534777	EF534943	EF534901	EF534858	EF534817
D	*Rhacodactylus ciliatus*	TG 00080	New Caledonia	—	EF534944	EF534902	EF534859	—
D	*Rhacodactylus ciliatus*	AMS 146595	Rivière Bleue, New Caledonia	EF534778	—	—	—	EF534818
D	*Oedura marmorata*	AMS 143861	Queensland, Australia	EF534779	EF534945	EF534903	EF534860	EF534819
C	*Nephrurus milii*	AMB 499	Western Australia, Australia	EF534780	EF534946	EF534904	EF534861	EF534820
C	*Carphodactylus laevis*	AMS 143258	Queensland, Australia	EF534781	EF534947	EF534905	EF534862	EF534821
Py	*Lialis burtonis*	TG 00078	Provinsi Papua, Indonesia	EF534782	EF534948	EF534906	EF534863	EF534822
Py	*Pygopus nigriceps*	AMB 53	Northern Territory, Australia	EF534783	EF534949	EF534907	EF534864	EF534823
S	*Sphaerodactylus roosevelti*	CAS 198428	Bahia de la Ballena, Puerto Rico	EF534785	EF534951	EF534909	EF534866	EF534825
S	*Gonatodes albogularis*	MVZ 204073	Limon, Costa Rica	EF534797	—	—	—	EF534839
S	*Gonatodes albogularis*	KU 289808	San Salvador, El Salvador	—	EF534965	EF534923	EF534880	—
S	*Saurodactylus brosseti*	TG 00082	Morocco	EF534802	EF534970	EF534928	EF534885	EF534844
S	*Teratoscincus roborowskii*	TG 00070	China	EF534799	EF534967	EF534925	EF534882	EF534841
S	*Aristelliger lar*	JB 01	Dominican Republic	EF534805	EF534973	EF534931	EF534888	EF534847
S	*Euleptes europaea*	No number	Liguria, Italy	EF534806	EF534974	EF534932	EF534889	EF534848
Ph	*Asaccus platyrhynchus*	CAS 227605	Wilayat Nazwa, Oman	EU293625	EU293715	EU293670	EU293647	EU293693
Ph	*Asaccus* sp.	JB 15	Mirbat, Oman	EU293626	EU293716	EU293671	EU293648	EU293694
Ph	*Haemodracon riebeckii*	JB 11	Socotra Island, Yemen	EU293627	EU293717	EU293672	EU293649	EU293695
Ph	*Homonota darwinii*	LJAMM 4601	Puerto Deseado, Santa Cruz, Argentina	EU293628	EU293718	EU293673	EU293650	EU293696
Ph	*Homonota fasciata*	TG 00085	Paraguay	EU293629	EU293719	EU293674	EU293651	EU293697
Ph	*Phyllodactylus tuberculosus*	KU 289758	PN El Imposible, Ahuachapán, El Salvador	EU293630	EU293720	EU293675	EU293652	EU293698
Ph	*Phyllodactylus bugastrolepis*	ROM 38489	Isla Santa Catalina, Baja California Sur, Mexico	EU293631	EU293721	EU293676	EU293653	EU293699
Ph	*Phyllodactylus reissii*	JB 39	Peru	EU293632	EU293722	EU293677	EU293654	EU293700
Ph	*Phyllodactylus xanti*	ROM 38490	Baja California Sur, Mexico	EF534807	EF534975	EF534933	EF534890	EF534849
Ph	*Phyllopezus maranjonensis*	ZFMK 84995	Balsas, Amazonas, Peru	EU293633	EU293723	EU293678	EU293655	EU293701
Ph	*Phyllopezus pollicaris przewalskii*	TG 00105	Paraguay	—	EU293724	EU293679	EU293656	—
Ph	*Phyllopezus pollicaris przewalskii*	YPM 13683	Paraguay	EU293634	—	—	—	—
Ph	*Phyllopezus pollicaris pollicaris*	MZUSP 92491	Parque Nacional da Serra das Confusões, Piauí, Brazil	EU293635	EU293725	EU293680	EU293657	EU293702
Ph	*Ptyodactylus guttatus*	TG 00072	Egypt	EU293636	EU293726	EU293681	EU293658	EU293703
Ph	*Ptyodactylus hasselquistii*	YPM 13609	Egypt	EU293637	EU293727	EU293682	EU293659	EU293704
Ph	*Tarentola chazaliae*	TG 00130	Morocco	EU293638	EU293728	EU293683	EU293660	EU293705
Ph	*Tarentola delalandii*	JB 43	Canary Islands	EU293639	EU293729	EU293684	EU293661	EU293706
Ph	*Tarentola gigas*	JB 45	Cape Verde Islands	EU293640	EU293730	EU293685	EU293662	EU293707
Ph	*Tarentola mauritanica*	TG 00129	Egypt	EU293641	EU293731	EU293686	EU293663	EU293708
Ph	*Thecadactylus rapicauda*	ENS 7108	Izabal, Guatemala	EU293642	EU293732	EU293687	EU293664	EU293709
Ph	*Thecadactylus rapicauda*	USNM 561446	St. Croix, U.S. Virgin Islands	EU293643	EU293733	EU293688	EU293665	EU293710
Ph	*Thecadactylus solimoensis*	KU 214929	Cuzco Amazonico, Madre de Dios, Peru	EU293644	EU293734	EU293689	EU293666	EU293711
G	*Narudasia festiva*	AMB 3243	Narudas, Namibia	EF534808	EF534976	EF534934	EF534891	EF534850
G	*Cnemaspis limi*	LLG 6267	Pulau Tioman, Malaysia	EF534809	EF534977	EF534935	EF534892	EF534851
G	*Rhoptropus boultoni*	CAS 214713	Twyfelfontein, Namibia	EF534810	EF534978	EF534936	EF534893	EF534852
G	*Chondrodactylus bibronii*	JV1850	30 km N Swakopmund, Namibia	EU293645	EU293735	EU293690	EU293667	EU293712
G	*Pachydactylus punctatus*	AMB 8311	Farm Celine, Limpopo Prov., South Africa	EU293646	—	—	—	EU293713
G	*Pachydactylus punctatus*	AMB 8312	Farm Celine, Limpopo Prov., South Africa	—	EU293736	EU293691	EU293668	—
G	*Paroedura picta*	FG/MV 2002.B1	Berenty, Madagascar	EF536149	EU293737	EU293692	EU293669	EF536173
G	*Phelsuma madagascariensis*	FG/MV 2002.797	Manongarivo, Madagascar	EF534811	EF534979	EF534937	EF534894	AB081507
G	*Lepidodactylus lugubris*	AMB 4111	Kirimati, Kiribati	EF534812	EF534980	EF534938	EF534895	EF534853
G	*Gekko gecko*	No ID	unknown	EF534813	—	—	—	EF534854
G	*Gekko gecko*	TG 00079	Indonesia	—	EF534981	EF534939	EF534896	—
G	*Hemidactylus frenatus*	TG 00088	Indonesia	—	EF534982	EF534940	EF534897	—
G	*Hemidactylus frenatus*	AMB 7411	Pidenipitiya, Sri Lanka	EF534814	—	—	—	EF534855
—	*Tiliqua rugosa*	JFBM 13685	New South Wales, Australia	EF534815	EF534983	EF534941	EF534898	EF534856
—	*Rhineura floridana*	FLMNH 141814	Florida, USA	AY662618	DQ119631	AY487347	EF534899	EU29371

Table 2 PCR and sequencing primers used in this study.

Primer name	Primer sequence (5′ to 3′)	Source
RAG1		
G396	TCTGAATGGAAATTCAAGCTGTT	Groth & Barrowclough (1999)
G397	AAAGGTGGCCGACCGAGGCAGCATC	Groth & Barrowclough (1999)
F700	GGAGACATGGACACAATCCATCCTAC	Bauer *et al.* (2007)
R700	TTTGTACTGAGATGGATCTTTTTGCA	Bauer *et al.* (2007)
RAG2		
EM1-F	TGGAACAGAGTGATYGACTGCAT	Gamble *et al.* (2008a)
EM1-R	ATTTCCCATATCAYTCCCAAACC	Gamble *et al.* (2008a)
PY1-F	CCCTGAGTTTGGATGCTGTACTT	Gamble *et al.* (2008a)
PY1-R	AACTGCCTRTTGTCCCCTGGTAT	Gamble *et al.* (2008a)
c-mos		
G73	GCGGTAAAGCAGGTGAAGAAA	Saint *et al.* (1998)
G74	TGAGCATCCAAAGTCTCCAATC	Saint *et al.* (1998)
FU-F	TTTGGTTCKGTCTACAAGGCTAC	Gamble *et al.* (2008a)
FU-R	AGGGAACATCCAAAGTCTCCAAT	Gamble *et al.* (2008a)
ACM4		
Tg-F	CAAGCCTGAGAGCAARAAGG	Gamble *et al.* (2008a)
Tg-R	ACYTGACTCCTGGCAATGCT	Gamble *et al.* (2008a)
Int–F	TTTYCTGAAGAGCCCTCTGGTC	Gamble *et al.* (2008b)
Int–R	CAAATTTCCTGGCAACATTRGC	Gamble *et al.* (2008b)
PDC		
PHOF2	AGATGAGCATGCAGGAGTATGA	Bauer *et al.* (2007)
PHOR1	TCCACATCCACAGCAAAAAACTCCT	Bauer *et al.* (2007)

PCR conditions for RAG1 and PDC are detailed in Greenbaum *et al*. (2007b). We purified PCR products using Exonuclease I and Shrimp Alkaline Phosphatase (Hanke & Wink 1994), the QIAquick PCR Purification kit (Qiagen), or AMPure magnetic bead solution (Agencourt Bioscience, Beverly, MA) following the manufacturer's recommendations. Sequencing was performed using Big Dye (Perkin Elmer, Boston, MA) or DYEnamic™ ET Dye Terminator Kit (GE Healthcare. Little Chalfont, UK) terminator cycle sequencing with CleanSeq magnetic bead solution purification (Agencourt Bioscience) on an ABI 3730xl at the Advanced Genetic Analysis Center, University of Minnesota, or an ABI 3700 automated sequencer at Villanova University. All PCR reactions were run with negative controls. Sequences were assembled using Sequencher 4.2 (Gene Codes, Ann Arbor, MI). We aligned sequences using T-Coffee (Notredame *et al*. 2000) and all sequences were translated to amino acids using MacClade 4.08 (Maddison & Maddison 1992) to confirm alignment and gap placement.

We analysed each gene partition individually, as well as the concatenated data, using maximum parsimony. We conducted parsimony analyses using heuristic search algorithms in PAUP*4.0b10 (Swofford 2002), employing equally weighted and unordered characters and tree bisection–reconnection branch swapping. Multistate data were treated as polymorphisms and gaps treated as missing. Nonparametric bootstraping (Felsenstein 1985), using 1000 pseudoreplicates, was performed to assess nodal support.

We analysed the concatenated data set, and each gene individually, using maximum likelihood with the program GARLI 0.951 (Zwickl 2006). Analyses were automatically terminated after 10 000 generations without an improvement in topology. Nodal support was evaluated using 100 bootstrap pseudoreplicates (Felsenstein 1985) with each repetition terminated after 5000 generations without a topology improvement. We used GTR + I + Γ model, as determined using the Akaike Information Criterion (AIC) in MRMODELTEST 2.2 (Nylander 2004), with model parameters estimated.

We conducted Bayesian phylogenetic analyses of the combined data set using MRBAYES 3.1.2 (Huelsenbeck & Ronquist 2001). Analyses were initialized with a random starting tree and run for 4 000 000 generations with sampling every 100 generations. Convergence was checked by importing the MRBAYES output to the program TRACER v1.3 <http://evolve.zoo.ox.ac.uk/beast/>, which plots the likelihood values by generation. 'Burn in' trees (5000) were discarded and the remaining samples were used to estimate the posterior probability values, branch lengths and topology. We used the AIC, as implemented in MRMODELTEST 2.2 (Nylander 2004), to estimate the best-fit model of nucleotide substitution for each data partition. We used Bayes factors to determine the most appropriate data partitioning strategy following the methods of Nylander *et al*. (2004) and Brandley *et al*. (2005). We considered hypotheses with 2 ln Bayes factors with a value > 10 as being very strongly supported (Kass & Raftery 1995). We examined four different data partitioning strategies: all data combined (1 partition), data partitioned by gene (5 partitions), data partitioned by codon (3 partitions) and data partitioned by codon for each gene individually (15 partitions).

We tested alternative phylogenetic hypotheses in a likelihood framework using the SH test (Shimodaira & Hasegawa 1999). The SH test was conducted in PAUP*4.0b10 (Swofford 2002) with 1000 RELL bootstraps. We considered two alternative hypotheses: monophyly of the genera *Pachydactylus*, *Chondrodactylus*, *Rhoptropus* and *Tarentola*, a clade diagnosed by hyperphalangy of the first digit of the manus and pes (Russell 1972; Haacke 1976; Kluge 1987; Kluge & Nussbaum 1995; Lamb & Bauer 2006); and monophyly of Gekkonini (Kluge 1983), a clade diagnosed by the absence of the second ceratobranchial arch.

Results

We obtained sequence data for all taxa and genes except PDC for the gecko *Phyllopezus p. przewalskii*. Multiple individuals of the same species were sequenced for different loci in some instances; these are noted in Table 1. Of the 2643 characters, 453 characters were variable but not parsimony informative

Table 3 Estimated models of sequence evolution, total number of characters, number of variable sites and number of parsimony-informative sites for each data partition used in the phylogenetic analyses.

Partition	Model	No. of characters in partition	No. of variable sites	No. of parsimony-informative sites
All data	GTR + I + Γ	2643	453	1065
RAG2	GTR + I + Γ	365	60	133
c-mos	GTR + I + Γ	383	60	144
ACM4	GTR + I + Γ	447	51	149
RAG1	GTR + I + Γ	1053	237	460
PDC	GTR + I + Γ	395	45	179
1st codon	GTR + I + Γ	881	141	264
2nd codon	GTR + I + Γ	881	135	197
3rd codon	GTR + Γ	881	177	604
RAG2 1st codon	HKY + Γ	121	17	32
RAG2 2nd codon	GTR + Γ	122	17	20
RAG2 3rd codon	HKY + Γ	122	26	81
c-mos 1st codon	HKY + I	128	19	44
c-mos 2nd codon	GTR + Γ	128	16	23
c-mos 3rd codon	HKY + I	127	25	77
ACM4 1st codon	GTR + Γ	149	9	30
ACM4 2nd codon	HKY + I + Γ	149	13	17
ACM4 3rd codon	GTR + Γ	149	29	102
RAG1 1st codon	HKY + Γ	351	80	109
RAG1 2nd codon	GTR + Γ	351	81	104
RAG1 3rd codon	HKY + Γ	351	76	247
PDC 1st codon	GTR + Γ	132	16	49
PDC 2nd codon	GTR + Γ	131	8	33
PDC 3rd codon	HKY + Γ	132	21	97

and 1065 were parsimony informative. Best-fit models of nucleotide substitution, as determined by AIC, are shown in Table 3. Sequence alignment was unambiguous and insertion/deletions in these genes have been commented on elsewhere (Gamble *et al*. 2008a). A 3-bp deletion at position 152 of PDC is a synapomorphy for the new, trans-Atlantic gecko clade (Phyllodactylidae, see below).

Parsimony and maximum likelihood analyses of the individual genes were largely congruent although there was poor support overall for many nodes (Fig. 1). All genes recovered a monophyletic trans-Atlantic gecko clade (Phyllodactylidae) consisting of *Ptyodactylus*, *Asaccus*, *Haemodracon*, *Tarentola*, *Thecadactylus*, *Phyllodactylus*, *Phyllopezus* and *Homonota*, sister to the remaining Gekkonidae. The combined data analyses provided stronger support and resolution across the tree than the individual gene trees. Monophyly of Gekkota was well supported with relation to outgroups (Fig. 2). The placement of the Eublepharidae as sister to the Gekkonidae + Phyllodactylidae + Sphaerodactylidae and the basal position of the Diplodactylidae + Carphodactylidae + Pygopodidae are consistent with other recent molecular gekkotan phylogenies (Donnellan *et al*. 1999; Han *et al*. 2004; Townsend *et al*. 2004; Gamble *et al*. 2008a). As with the single gene analyses, we recovered a well-supported trans-Atlantic gecko clade (Phyllodactylidae) forming a clade with the remaining Gekkonidae. Character support for higher-level clades is shown in Table 4.

Table 4 The number of base pair changes that support higher-level Gekkotan clades, calculated using maximum parsimony, partitioned by locus.

	RAG2	c-mos	ACM4	RAG1	PDC
Carphodactylidae + Diplodactylidae + Pygopodidae	7	3	9	34	10
Carphodactylidae	6	5	9	20	13
Diplodactylidae	1	3	2	5	11
Pygopodiddae	3	4	9	13	12
Eublepharidae + Sphaerodactylidae + Phyllodactylidae + Gekkonidae	0	3	0	7	0
Eublepharidae	10	8	5	12	6
Sphaerodactylidae + Phyllodactylidae + Gekkonidae	1	5	3	13	2
Sphaerodactylidae	5	1	2	3	3
Phyllodactylidae + Gekkonidae	4	3	4	8	2
Phyllodactylidae	2	3	3	6	3
Gekkonidae	2	1	2	9	1

The maximum likelihood tree from the combined data was significantly better, according to the SH test, than trees constrained to reflect alternative hypotheses. The combined data maximum likelihood tree had a –ln L score = 25036.42859. The first alternative hypothesis, which constrained a monophyletic *Pachydactylus*, *Chondrodactylus*, *Rhoptropus* and *Tarentola*, based on hyperphalangy of the first digit, had a –ln L score = 25178.56329 (difference in –ln L = 142.13469; $P < 0.001$). The second alternative hypothesis, which enforced monophyly of Gekkonini (*sensu* Kluge 1983), based on the absence of the second ceratobranchial arch, had a –ln L score = 25315.35999 (difference in –ln L = 278.93139; $P < 0.001$).

Discussion

Our analyses recovered a novel, trans-Atlantic clade (Phyllodactylidae) of geckos as the sister group to the remaining Gekkonidae *sensu* Gamble *et al*. (2008a). This novel clade was recovered in all analyses. The presence of a 3-bp deletion in PDC increases our confidence in the validity of this clade as indels and other rare genomic events are usually quite reliable as phylogenetic markers (Lloyd & Calder 1991; Van Dijk *et al*. 1999). The existence of this clade is unexpected, as no other phylogenetic or taxonomic hypotheses have proposed linking taxa represented in the Phyllodactylidae. In fact, previous authorities have consistently grouped constituent taxa with other genera outside of the Phyllodactylidae or in separate higher-level categories. In the Introduction, we

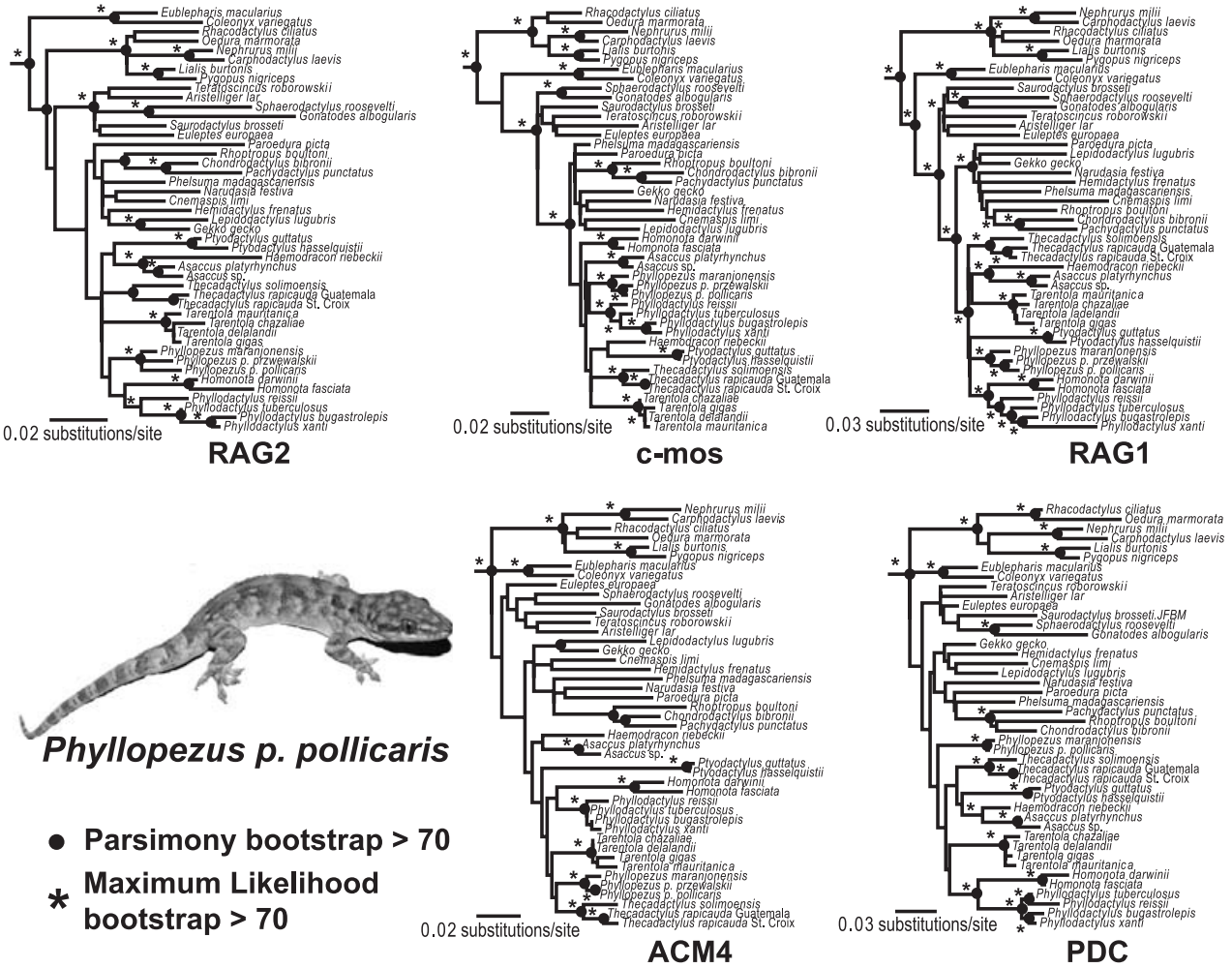

Fig. 1 Maximum likelihood phylogenies for each gene fragment analysed separately. Maximum parsimony (MP) and maximum likelihood (ML) bootstrap values are indicated. Photo by T. Gamble.

mentioned two reasons why such novel, higher-level clades might remain unknown. Below we discuss these reasons in more detail and demonstrate how they contributed to the failure of past workers to recover the Phyllodactylidae.

Taxon sampling

The examples mentioned in the introduction, Animalia, Mammalia and Squamata, like Gekkota, are species rich and geographically widespread. Any hope of accurate phylogenetic reconstruction requires sampling as diversely and thoroughly as possible (Hillis 1996, 1998; Graybeal 1998; Hedtke *et al.* 2006). Previous phylogenetic studies of geckos at the intergeneric level have largely focused on restricted geographical areas such as Africa and Madagascar (Joger 1985; Bauer 1990b; Kluge & Nussbaum 1995), Australia and Oceania (Donnellan *et al.* 1999), China (Han *et al.* 2001) and South America (Abdala & Moro 1996) or on putatively monophyletic groups (Kluge 1976; Grismer 1988; Bauer 1990a; Kluge 1995; Ota *et al.* 1999; Jennings *et al.* 2003). Those studies that did have broader taxonomic sampling (e.g. Underwood 1954; Kluge 1983, 1987), as we discuss below, suffered from the other major impediment to recovery of novel higher-level clades such as Phyllodactylidae, character homoplasy and lack of phylogenetic resolution due to reliance on a few, superficially similar morphological features.

Morphology

The reliance on too few morphological features is a serious concern in phylogenetic reconstruction (Scotland *et al.* 2003). To illustrate this we mapped pedal morphology ('naked' toed or padless, basal pads or terminal, leaf-like pads), phalangeal formula (hyperphalangic or not), and presence or absence of the

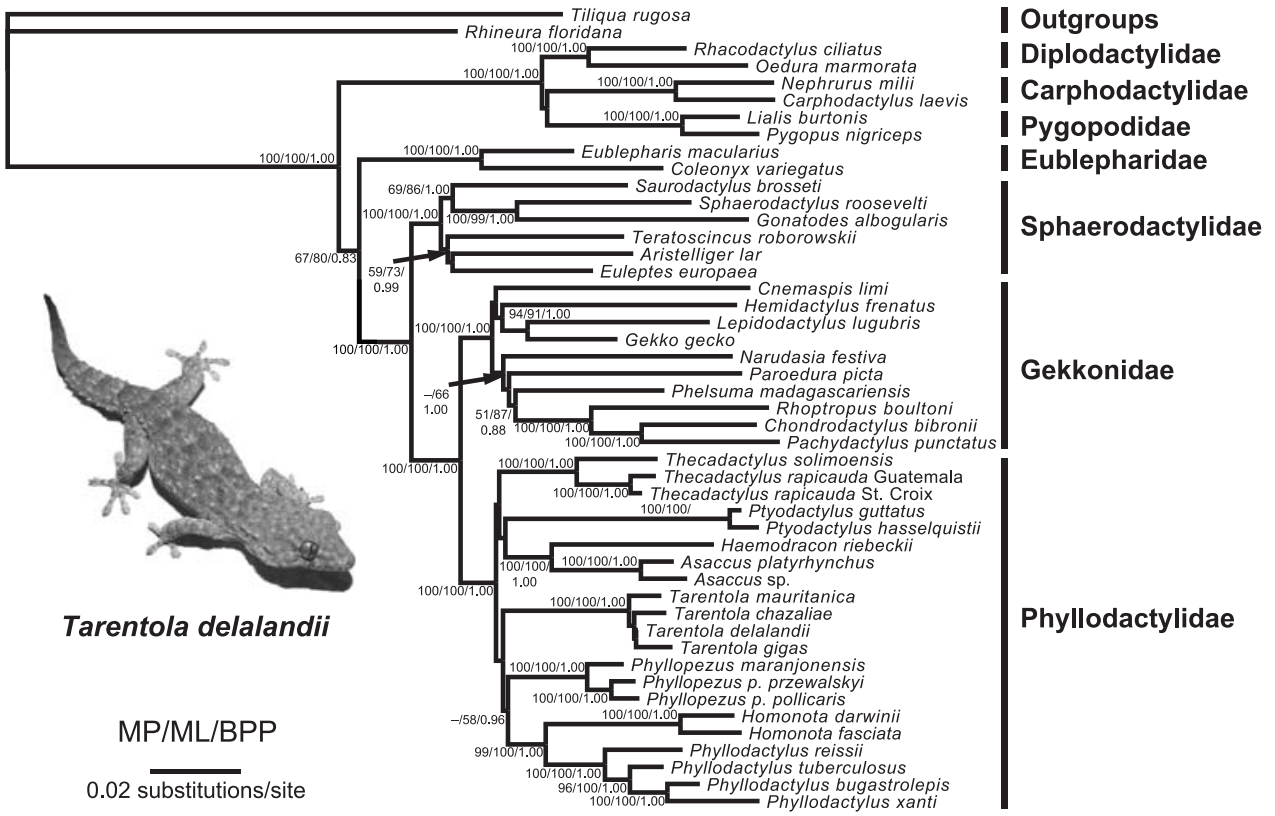

Fig. 2 Maximum likelihood phylogeny for combined data. Maximum parsimony (MP) and maximum likelihood (ML) bootstrap values as well as Bayesian posterior probabilities (BPP) are indicated. Clade names are shown on the right. Photo by T. Gamble.

second ceratobranchial arch onto the maximum likelihood tree illustrating the complexity of these character distributions among sampled gekkotan taxa (Fig. 3). We did not conduct ancestral state reconstructions because of incomplete taxon sampling outside the Phyllodactylidae. Important historical treatments of gecko taxonomy and systematics often used external digital morphology as the sole or primary basis for assigning genera to higher-level groupings (e.g. Fitzinger 1843; Boulenger 1885; Loveridge 1947). Herpetologists have long recognized that characters, such as digital morphology, are rife with homoplasy and convergence, and have been apprehensive of using digital characters as evidence of relationship and descent (Dixon & Kroll 1974; Russell 1976, 1979). Other morphological characters, unfortunately, have offered little in the way of additional phylogenetic resolution within Gekkoninae *sensu* Kluge (1987). This is not an indictment of morphological data per se (Wiens 2004), but rather a reflection of the fact that a relatively small set of characters have, heretofore been used to reconstruct gekkotan phylogeny and/or to allocate taxa to higher order groups.

Constituent genera of the Phyllodactylidae have previously been grouped with other taxa, often based on single, sometimes homoplasious, characters. The sampled genera *Asaccus*, *Euleptes*, *Haemodracon*, *Paroedura* and *Phyllodactylus*, for example, were at one time grouped together in the genus *Phyllodactylus* based on their 'leaf-toed' digital morphology (Dixon & Kroll 1974; Kluge 1983; Bauer *et al*. 1997). The distribution of the 'leaf-toed' morphology, largely defined as digits with broad, divided, terminal scansors, appears to have evolved independently several times in Gekkota (Fig. 3, Russell 1972; Dixon & Kroll 1974; Kluge 1983; Bauer *et al*. 1997; Jackman *et al*. 2008). Other members of the Phyllodactylidae have been associated with taxa in higher-level clades based on digital morphology. Vanzolini (1968) suggested affinities between *Hemidactylus*, *Briba*, *Bogertia* and *Phyllopezus* based on proximal, digital adhesive pads with compressed, elongated phalanges. Although Vanzolini (1968) realized the problems that afflicted Gekkotan systematics and the use of 'trivial characters' to define genera, he was nonetheless convinced of the close relationships among these four genera, even going so far as to suggest that they may be congeneric. We did not include *Bogertia* in the current study but *Briba* and *Hemidactylus* are in fact closely allied (Carranza & Arnold 2006) and our data do not support a close relationship between *Phyllopezus*

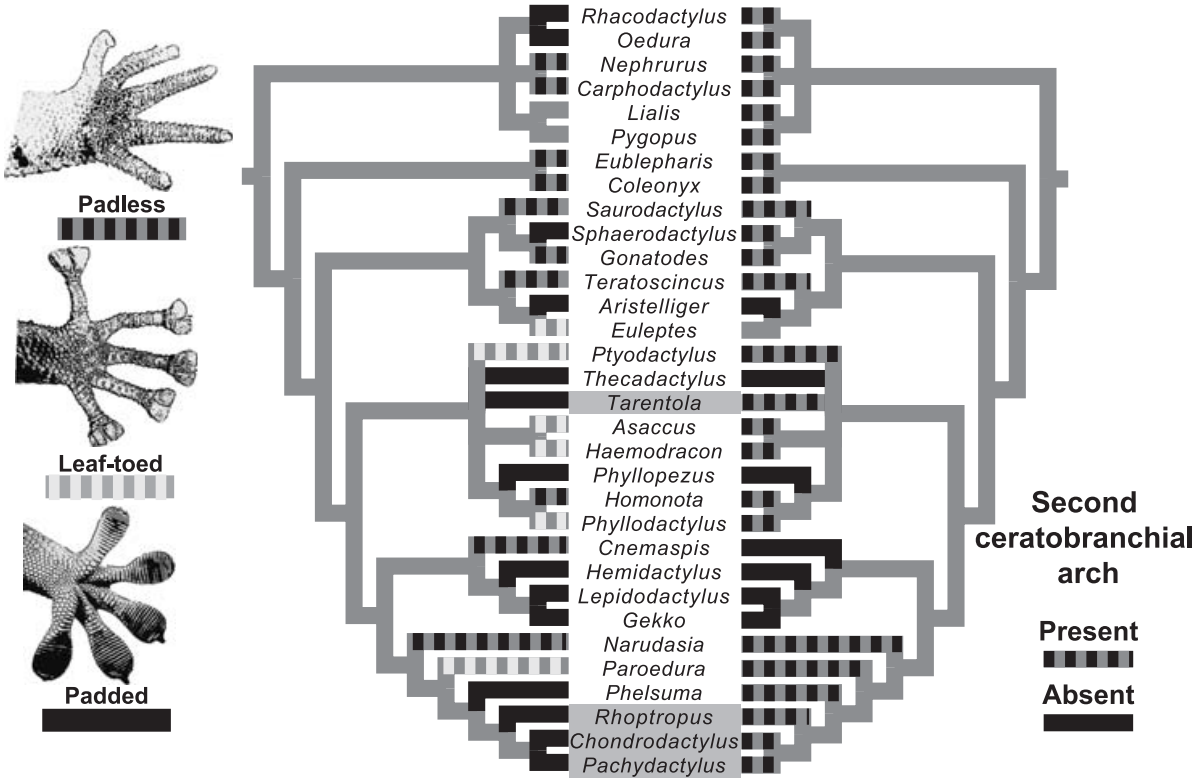

Fig. 3 Simplified topology from our data showing the distribution of the following characters: gecko digital structure, on left; hyperphalangy, shaded generic names; and the presence/absence of the second ceratobranchial arch, on right. These distributions do not show or imply the character states of ancestral taxa. Images depict feet of *Homonota fasciata* (padless), *Phyllodactylus tuberculosus* (leaf-toed) and *Tarentola annularis* (padded). Photos by T. Gamble.

and *Hemidactylus*. Abdala & Moro (1996) considered *Homonota*, *Phyllodactylus* and *Hemidactylus* to be closely related based on cranial musculature. While our phylogeny clearly places *Hemidactylus* in the Gekkonidae we did recover a close relationship between *Homonota* and *Phyllodactylus*.

The second ceratobranchial arch offers another example of character homoplasy across Gekkota. Kluge (1983) used the loss of this structure to define the Gekkonini, a clade within his Gekkoninae, a subfamilial rank which corresponds to our Gekkonidae + Phyllodactylidae and certain lineages within Sphaerodactylidae. Like digital morphology, the absence of the second ceratobranchial arch is homoplasious and appears to have been lost independently several times within Gekkota (Bauer 1990b; Han *et al*. 2004; Fig. 3).

The third example of convergence, hyperphalangy, may be the most extraordinary. Phalangeal losses are common in many tetrapod lineages, but additions are extremely rare, occurring chiefly in fully aquatic taxa in which the digits become elongate into flipper-like structures (Romer 1956). Phalangeal gains in squamates are known only from one skink and one agamid species and representatives of six gekkotan genera (Greer 1992; Russell & Bauer in press). Among these taxa, the particular expression of hyperphalangy is typically unique to a single taxon; thus the convergent hyperphalangy of *Tarentola* and the *Pachydactylus* group is particularly remarkable.

The trans-Atlantic distribution of Phyllodactylidae species is similar to the distribution of another Gekkotan clade, the Sphaerodactylidae (Gamble *et al*. 2008a). What makes the distributions of these two gecko clades different from most other Gondwanan distributed taxa is that their Old World component is restricted to Northern Africa, the Arabian Peninsula, and central and southern Asia. This stands in sharp contrast to groups such as pelomedusoid turtles, for example, where the Old World taxa occur in sub-Saharan Africa and Madagascar (Bauer 1993; Noonan 2000). On the other hand, the New World components of the Sphaerodactylidae have a predominantly Caribbean and Guiano–Amazonian distribution, whereas those of the Phyllodactylidae are chiefly distributed from the Amazon southwards. Overlap with sphaerodactylids occurs with some representatives of *Phyllodactylus* and *Thecadactylus* in Amazonian South America, Central America and some Caribbean islands while the sphaerodactylid genus

Coleodactylus overlaps with *Gymnodactylus* and *Phyllopezus* in cerrado and caatinga habitats in central Brazil.

Taxonomy

We name this clade Phyllodactylidae. This name is formed as a traditional Linnaean family name and would be a name of this rank in the Linnaean hierarchy. Dixon & Kroll (1974) used the term 'phyllodactyline' as an adjective to refer to a subset of 'leaf-toed' geckos but did not formally propose a taxonomic group 'Phyllodactylinae'. Phyllodactylidae is here defined as the crown clade consisting of all geckos sharing a more recent common ancestor with *Phyllodactylus pulcher* Gray 1828 than with *Gekko* (originally *Lacerta*) *gecko* Linnaeus 1758. At present, the sole defining synapomorphy of the group is the 3 bp deletion in PDC.

Composition: approximately 103 species in the following genera: *Phyllodactylus* Gray (47 species); *Phyllopezus* (3 species); *Homonota* (8 species); *Asaccus* (9 species); *Ptyodactylus* (6 species); *Thecadactylus* (2 species); *Haemodracon* (2 species); and *Tarentola* (19 species). We predict that the South American endemic genera *Gymnodactylus* (4 species), *Garthia* (2 species) and *Bogertia* (monotypic) will also be members of this group. All other genera of gekkotans have been excluded from membership on the basis of molecular phylogentic results or possession of multiple unambiguous morphological synapomorpies of other clades. Russell & Bauer (1988, 1990) provided data from paraphalangeal and digital structure suggesting that *Bogertia* was allied to *Thecadactylus* and *Phyllopezus*. Abdala & Moro (1996) found support for a relationship between *Bogertia* and *Thecadactylus* based on cranial myology, whereas Abdala (1996) found cranial osteological characters to unite *Bogertia* with *Phyllopezus*. Bauer *et al*. (1997) noted the absence of cloacal sacs and bones in *Haemodracon* and *Asaccus*, and in retrospect, this character supports the sister group relationship of these two genera, one of the only intergeneric patterns in the Phyllodactylidae that has strong support. Single-egg clutches, which were identified as a possible derived feature for the Sphaerodactylidae (Gamble *et al*. 2008a), may be synapomorphic at some level within this clade as well. Single-egg clutches are common among the Phyllodactylidae, typifying most *Phyllodactylus* (Dixon & Huey 1970), all *Asaccus* (Arnold & Gardner 1994) and *Thecadactylus* (Lee 1996) and variably occurring within species of *Gymnodactylus*, *Homonota* and *Ptyodactylus* (Schleich *et al*. 1996; Rösler 2005).

Although previous higher-order analyses sampled too poorly or lacked sufficient data to recover Phyllodactylidae, in retrospect, evidence for some of the intergeneric groupings had been identified. For example, Joger (1984, 1985), using immunological methods, identified *Ptyodactylus* as the closest relative of *Tarentola*, and Han *et al*. (2004) recovered a *Phyllodactylus* + *Tarentola* clade (albeit with poor support) in their MP analysis of c-mos data.

There are obvious similarities between the identification of cryptic species and novel or 'cryptic' higher-level taxa, as mentioned in the introduction, but there are also important differences. The poor performance of morphology in identifying cryptic species relates to the conservative morphologies of closely related taxa, since sister species will likely share most traits (Zink & McKitrick 1995; Egge & Simons 2006). The failure to recover higher-level taxa, on the other hand, is due primarily to issues related to phylogenetic reconstruction and morphological specialization. 'Cryptic' higher-level taxa, with further research, may be diagnosable using morphology. Recent publications, for example, have revealed potential synapomorphies for Afrotheria by critically re-examining mammalian vertebral morphology and by using a novel source of characters related to the placenta and foetal membranes (Mess & Carter 2006; Sánchez-Villagra *et al*. 2007). A similar effort to discover new sources of morphological characters for geckos would likely uncover synapomorphies for Phyllodactylidae and other major gekkotan clades. It stands to reason that as large-scale phylogenetic research progresses through so-called 'tree of life' projects that additional novel, higher-level taxa will be identified from genetic data. It is important that new morphological data sets continue to be developed to keep pace with the molecular phylogenetic research to better understand morphological character evolution and maintain a practical link between morphology and taxonomy.

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