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

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# Abstract

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.

# 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**.

**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.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Family** | **Species** | **Specimen ID** | **Locality** | **Genbank accession numbers** |  |  |  |  |
|  |  |  |  | **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 |

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. 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.

**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) |

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.

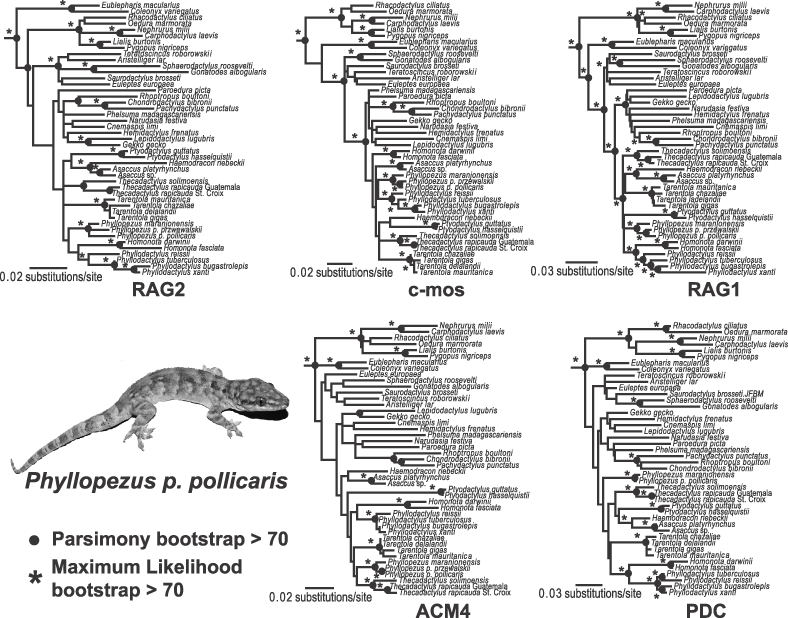
# Results

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 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).

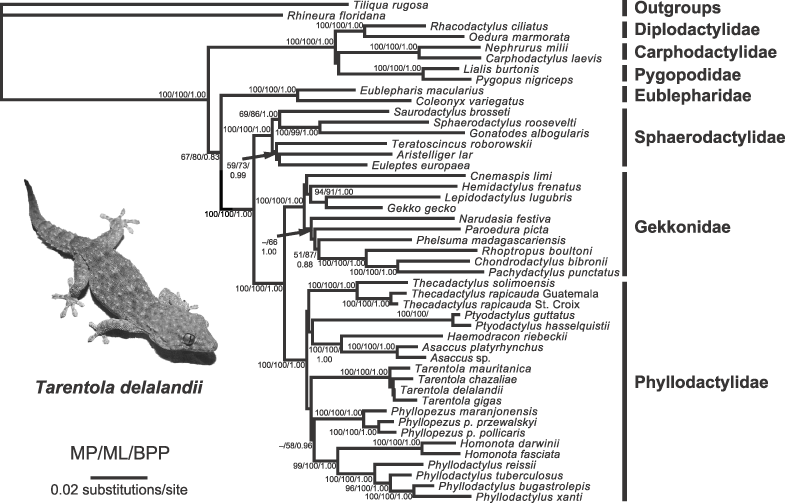
**Table 3.**Estimated models of sequence evolution, total number of characters, number of variable sites and number of parsimony-informative sites for each data partition used in the phylogenetic analyses.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **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 |

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**.

[](https://onlinelibrary.wiley.com/cms/asset/b67e479e-7600-4024-8554-f226fd3d0d7c/zsc_330_f1.gif)

**Figure 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.

[](https://onlinelibrary.wiley.com/cms/asset/e6fd54ba-c66d-4907-95f0-4ae4055b04cb/zsc_330_f2.gif)

**Figure 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.

**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

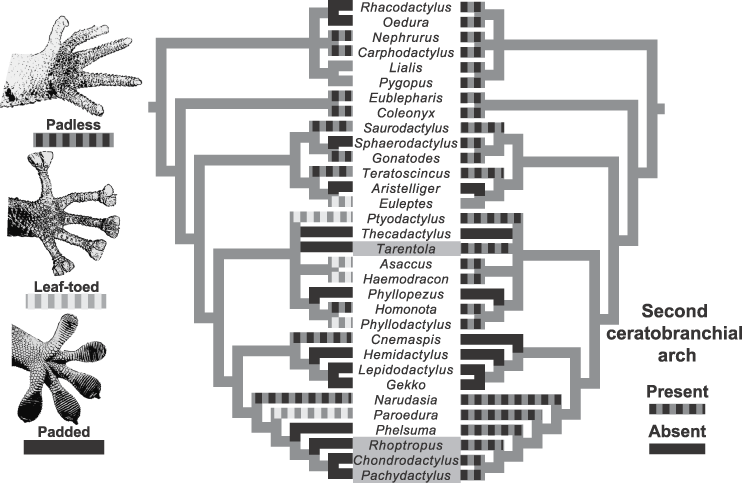
Our analyses recovered a novel, trans-Atlantic clade (Phyllodactylidae) of geckos as the sister group to the remaining Gekkonidae *sensu***Gamble *et al*. (2008a**). This novel clade was recovered in all analyses. The presence of a 3-bp deletion in PDC increases our confidence in the validity of this 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 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 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.

[](https://onlinelibrary.wiley.com/cms/asset/9174b63b-3e4e-4d8a-a79d-1f545aa1b6e9/zsc_330_f3.gif)

**Figure 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.

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* 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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