

FORUM

HERPETOLOGICAL JOURNAL, Vol. 14, pp. 51-53 (2004)

**TORTOISE SYSTEMATICS: A
CRITIQUE OF A RECENT PAPER BY
VAN DER KUYL *ET AL.* (2002)**

JARMO PERÄLÄ

*Ecological Genetics Research Unit, Department of
Ecology and Systematics, University of Helsinki, Finland*

Key words: nomenclature, taxonomy, Testudines, phylogeny

An article by van der Kuyl *et al.* (2002) on tortoise systematics published recently in *Molecular Phylogenetics and Evolution* [MPE] demands critical discussion. My written criticism, submitted initially for publication in MPE's *Letters to the Editor* section, was rejected by the editor on the grounds that "...it mainly criticizes formal taxonomic practices of van der Kuyl *et al.* (2002) rather than providing noteworthy reinterpretations of molecular evolutionary issues". Thus, it seems that MPE is prepared to publish papers on phylogenetic issues in zoology even if such works fail to comply with the International Code of Zoological Nomenclature (ICZN, 1999) [henceforth the Code]. Consequently, and also because of the independently unverifiable origins of the materials used, it is currently almost impossible to interpret the results as published in van der Kuyl *et al.* (2002). In other words, the paper in question does not allow reinterpretation of evolutionary issues because of the unorthodox nomenclature and presentation used. For this critique, names of taxa as used by van der Kuyl *et al.* (2002) are indicated with quotation marks ["..."].

General comments. The authors (van der Kuyl *et al.*, 2002) used mitochondrial 12S rRNA gene sequences to test variation and phylogenetic relationships among Mediterranean and Central Asian tortoises, that is, the genus *Testudo* Linnaeus 1758 *sensu lato* (in the sense of Lapparent de Broin, 2000, 2001; among others). Sampled testudinid taxa also included representatives of *Geochelone* Fitzinger 1835, *Chelonoidis* Fitzinger 1835, and *Indotestudo* Lindholm 1929. Maximum likelihood and neighbour-joining analyses yielded close to identical tree topologies. Two major lineages within "*Testudo*" comprise (a) "*T. graeca*", "*T. marginata*" and "*T. kleinmanni*" and, (b) "*T. hermanni*", "*T. horsfieldi*" and "*Indotestudo elongata*", according to the authors. Van der Kuyl *et al.* (2002) state that maximum parsimony analysis supports the first clade but not the second. However, the published phylogenies pertaining to testudinids – apart from their monophyly – generally

have very low bootstrap support and are thus largely unresolved, a point the authors fail to address. Haplotype variation is said to be greater in "*T. graeca*" than in "*T. hermanni*". Despite the interesting scope of the article, it seems likely that the reviewers of this particular paper were molecular biologists exclusively, rather than taxonomists, and that no experts in chelonian systematics were consulted before acceptance and publication.

Multiple references to earlier literature in van der Kuyl *et al.* (2002) appear without an actual citation, as if the published inferences were those of the authors themselves [e.g., "...six species are currently recognized in the genus *Testudo*"]. On the other hand, several relevant references to testudinid systematics from within the past ten to twenty years (including the erection of new taxa: Chkhikvadze & Tuniyev, 1986; Weissinger, 1987; Chkhikvadze, 1988; Chkhikvadze *et al.*, 1990; Chkhikvadze & Bakradze, 1991; Pieh, 2001a; to name a few) are omitted without explanation, or citations refer to second-hand sources (e.g. "Ernst & Barbour, 1989b", with reference to the resurrection of *Indotestudo*). Problematic taxa erected or resurrected over a decade ago by Martin and/or Highfield, the validity of which have been debated at least since the publication of Iverson (1992), are taken seemingly at face value by van der Kuyl *et al.* (2002). Generalized, and incorrect or contradictory, statements are made without empirical proof, particularly relative to geographical distributions, and supposed human introductions, of taxa. Some species group names are attached to geographical populations irrespective of their correct use and/or previously published literature [see *Specifics* below]. Apart from being very short (*circa* 400 nucleotides only), the gene sequence used is probably too conservative for inferring taxonomically meaningful variation in testudinids at species level [meaning subspecific level in the sense of the authors], because mitochondrial 12S rRNA partly fails to mirror major structural differences which would be found easily by the application of morphological methodologies (e.g. Perälä, 2002). Other – and preferably multiple – genes might be more suitable for this task. In addition, "areas of difficult alignment" (van der Kuyl *et al.*, 2002) – which could potentially contain relevant information – were excluded from the analysis. Of quickly evolving mitochondrial gene sequences, cytochrome *b* (Lenk *et al.*, 1998, 1999; Feldman & Parham, 2002) and additionally adjacent tRNAs and ND4 (Feldman & Parham, 2002) have been shown to be relatively informative at the species level in other chelonians. For example, cytochrome *b* was more informative than 12S rRNA regarding genetic variation in *T. graeca* Linnaeus 1758 from Morocco and Spain (Álvarez *et al.*, 2000). Only subsequent analyses will show which genes, and whether mitochondrial or nuclear, are most useful in reconstructing the phylogenetic history of Mediterranean and Central Asian tortoises – a history that should be reflected in classifications.

Specifics. It is not clear why some allopatric populations classified initially under the same taxon and

Correspondence: J. Perälä, Ecological Genetics Research Unit, Department of Ecology and Systematics, PO Box 65 (Biocenter 3, Viikinkaari 1), FIN-00014 University of Helsinki, Finland. E-mail: jarmo.perala@helsinki.fi

with demonstrated 12S rRNA divergence were included in the phylogenetic analysis (African "*T. graeca*" including "*T. whitei*") by van der Kuyl *et al.* (2002), but others were not: sequences derived from some of the investigated populations of "*T. hermanni*" and some European "*T. graeca*" were excluded, while others were included. The selected outgroup taxa (*Emys* Duméril 1806 and *Trachemys* Agassiz 1857 [both Emydidae Rafinesque 1815]; and *Cuora* Gray 1855 [Geoemydidae Theobald 1868]) might additionally be suboptimal for the purpose. It is possible that these taxa are phylogenetically too distant in relation to the ingroup to be of practical use in character polarization. The use of other more closely related testudinids in the outgroup should have been the logical choice. More dramatically, the paper displays a lack of understanding of fundamental taxonomic principles, such as the fact that scientific names are permanently attached to type specimens which determine type localities and the subsequent use of nomenclature for a given population, not to mention other basic taxonomic applications as regulated by the Code (ICZN, 1999). This should be unacceptable in a paper dealing with biological systematics. It is very hard to interpret what van der Kuyl *et al.* (2002) actually meant by some of the taxon names (they were sometimes misspelled, such as "*chelonoides*" for *Chelonoidis* Fitzinger 1835), which appear with (though often incorrect) or without authority and date. Although (part of) the mentioned sequence data are deposited with GenBank, it does not help the interpretation of the results that no independently verifiable data are provided for the geographical origins of the samples; that is, references to voucher specimens (and their physical location) with accompanying detailed locality information. The importance of being able to simultaneously analyse morphology and DNA data derived from exactly the same specimens is emphasized by Puerto *et al.* (2001).

As for confusing taxonomy, there are too many incorrect applications to be mentioned point by point. However, one notable failure is that the name "*Testudo whitei* (Highfield and Martin, 1989)", used presumably for *T. whitei* Bennett 1836, is applied to North African tortoises despite the earlier inference that the type of *T. whitei* (which the authors have not examined; Ballasina, pers. comm. 2002), does not correspond morphologically to any African testudinid, nor to the specimens attributed to *T. whitei* Bennett by Highfield & Martin (1989) (Bour in David, 1994; Perälä in Ernst *et al.*, 2000). It is also noteworthy that in their Table 1 (including its legend), the authors technically introduce - although probably unintentionally - three new names as follows (van der Kuyl *et al.*, 2002: 180):

(a) "*Testudo (graeca) whitei* ^a..."^d Proposed new species, formerly *T. graeca*" from Algeria. The name *Testudo graeca whitei* van der Kuyl *et al.* 2002 is a primary junior homonym of *T. whitei* Bennett 1836, and thus permanently invalid (ICZN, 1999: Art. 57.1). Furthermore, it is a *nomen nudum* because no type specimen was fixed and because the new name was not

accompanied by a description (ICZN, 1999: Articles 16.4, 72.3, and 13.1.1). In this context it is relevant to note that the type locality of *Testudo graeca* Linnaeus 1758 is in Santa Cruz, Oran, Algeria by designation of Strauch (1862), and that *T. graeca* is also the type species of *Testudo* Linnaeus 1758 by designation of Bell (1828).

(b) "*Testudo graeca Sardinia* ^a..."^a Proposed new subspecies" from Sardinia. The name *Testudo graeca sardinia* van der Kuyl *et al.* 2002 is unavailable (a *nomen nudum*) according to Articles 16.4, 72.3, and 13.1.1 (ICZN, 1999) because no holotype was designated and no description was provided.

(c) "*Testudo hermanni boettgeri*" from "Greece (Peloponnesus) ^a..."^a Proposed new subspecies." The name *Testudo hermanni boettgeri* van der Kuyl *et al.* 2002 is a primary junior homonym of *T. hermanni boettgeri* Mojsisovics 1889 and thus permanently invalid (ICZN 1999: Art. 57.1). It is also a *nomen nudum* because no type specimen was fixed and because the new name lacked a description (ICZN, 1999: Articles 16.4, 72.3, and 13.1.1).

It is also noteworthy that, the paper by van der Kuyl *et al.* (2002) was published later elsewhere with only cosmetic changes and with a reshuffled order of authors (Ballasina *et al.*, 2002). Because all of the mistakes described above for the article by van der Kuyl *et al.* (2002) were retained in Ballasina *et al.* (2002), including technical introductions of new names, these names, *Testudo graeca whitei* Ballasina *et al.* 2002, *Testudo graeca sardinia* Ballasina *et al.* 2002 and *Testudo hermanni boettgeri* Ballasina *et al.* 2002 (Ballasina *et al.*, 2002: 123, Table 1 including legend) are similarly unavailable for the same reasons. Additionally, the name "*Testudo graeca ibera*" is used inexplicably for Lebanese tortoises (Ballasina *et al.*, 2002: 123, Table 1).

The present criticism is a serious one because it is essential to address the matter sooner rather than later from the points of view of stable nomenclature, general editorial practice regarding evolutionary journals, and before mistakes are adopted into subsequent literature, or into conservation and animal-welfare policies. The latter policies might potentially legitimize introductions or translocations of individuals with identical molecular make-ups in the sense of van der Kuyl *et al.* (2002) - but without known origins or parentage [confiscations, captive stock] - into wild populations. This could have catastrophic effects on the genetic authenticity, as well as (due to ecological incompatibilities or pathology) for the general well-being of tortoise populations in the Mediterranean (Anonymous, 2001; Pieh, 2001b; Perälä, 2001), a region still certainly harbouring as yet formally undescribed tortoise diversity (Perälä, 2002).

Acknowledgements. I thank an anonymous reviewer for his comments which enhanced the final version of this critique.

Editor's Note: Van der Kuyl *et al.* were offered the opportunity to reply to Perälä's critique but declined to do so.

REFERENCES

- Álvarez, Y., Mateo, J. A., Andreu, A. C., Díaz-Paniagua, C., Dietz, A. & Bautista, J. M. (2000). Mitochondrial DNA haplotyping of *Testudo graeca* on both continental sides of the Straits of Gibraltar. *Journal of Heredity* **91**, 39-41.
- Anonymous (2001). DGHT-AG Schildkröten beschliesst einstimmig Resolution gegen unverantwortliche Auswilderung. *Elaphe* **9**, 37-38.
- Ballasina, D. L. Ph., van der Kuy, A. C., Dekker, J. T., Maas, J., Willemsen, R. E. & Goudsmit, J. (2002). Phylogenetic relationships among the species of the genus *Testudo* (Testudines: Testudinidae) inferred from mitochondrial 12S rRNA gene sequences. In *Chelonii* **3**, 117-129: *Actes du Congrès International sur le genre Testudo - 7 au 10 Mars 2001*. B. Fertard and B. Culorier (Eds.). Gonfaron, France: Editions SOPTOM.
- Bell, T. (1828). Characters of the order, families, and genera of the Testudinata. *Zoological Journal* **3**, 513-516.
- Chkhikvadze, V. M. (1988). Taxonomic status of modern land tortoise of Middle Asia and Kazakhstan [sic]. *News of the Academy of Sciences, Georgian S.S.R., Biological Series* **14**, 110-113. (In Russian)
- Chkhikvadze, V. M., Amiranashvili, N. G. & Ataev, C. H. (1990). New subspecies of tortoise from the south-western Turkmenistan [sic]. *News of the Science Academy of Turkmen S.S.R., Biological Series* **1**, 72-75. (In Russian)
- Chkhikvadze, V. M. & Bakradze, M. A. (1991). On the systematic position of the Recent land turtle from the Araxes Valley [sic]. *Trudy Tbilissk* **305**, 59-63: University of Tbilisi. (In Russian)
- Chkhikvadze, V. M. & Tuniyev, B. (1986). On the taxonomic status of modern land tortoise of the western Transcaucasus [sic]. *Bulletin of the Academy of Sciences of the Georgian S.S.R.* **123**, 617-620. (In Russian)
- David, P. (1994). Liste des reptiles actuels du monde. I. Chelonii. *Dumerilia* **1**, 1-127.
- Ernst, C. H., Altenburg, R. G. M. & Barbour, R. W. (2000). *Turtles of The World: World Biodiversity Database CD-ROM Series*. University of Amsterdam: Expert Center for Taxonomic Identification & Paris: UNESCO-Publishing.
- Feldman, C. R. & Parham, J. F. (2002). Molecular phylogenetics of Emydine turtles: Taxonomic revision and the evolution of shell kinesis. *Molecular Phylogenetics and Evolution* **22**, 388-398.
- Highfield, A. C. & Martin, J. (1989). *Testudo whitei* Bennett, 1836. New light on an old carapace – Gilbert White's Selborne tortoise re-discovered. *Journal of Chelonian Herpetology* **1**, 13-22.
- ICZN (1999). *International Code of Zoological Nomenclature*. Fourth Edition adopted by the International Union of Biological Sciences. London: ITZN.
- Iverson, J. B. (1992). *A Revised Checklist with Distribution Maps of the Turtles of the World*. Richmond, Indiana: Privately published.
- Lapparent de Broin, F. de, (2000). Les chéloniens de Sansan. In *La faune miocène de Sansan et son environnement*. Ginsburg, L. (Ed.). Mémoires du Muséum National d'Histoire Naturelle **183**, 219-261.
- Lapparent de Broin, F. de, (2001). The European turtle fauna from Triassic to the present. *Dumerilia* **4**, 155-217.
- Lenk, P., Fritz, U., Joger, U. & Wink, M. (1999). Mitochondrial phylogeography of the European pond turtle, *Emys orbicularis* (Linnaeus 1758). *Molecular Ecology* **8**, 1911-1922.
- Lenk, P., Joger, U., Fritz, U., Heidrich P. & Wink, M. (1998). Phylogeographic patterns in the mitochondrial cytochrome *b* gene of the European pond turtle (*Emys orbicularis*): first results. In *Mertensiella* **10**, 159-175: *Proceedings of the EMYS Symposium Dresden 96*. Fritz, U., Joger, U., Podlousky, R. & Servan, J. (Eds.). Rheinbach: DGHT.
- Perälä, J. (2001). A new species of *Testudo* (Testudines: Testudinidae) from the Middle East, with implications for conservation. *Journal of Herpetology* **35**, 567-582.
- Perälä, J. (2002). *The genus Testudo Linnaeus, 1758 sensu lato (Testudines: Testudinidae): Phylogeny, Taxonomy, Conservation*. PhD thesis, University of Bristol.
- Pieh, A. (2001a). *Testudo graeca soussensis*, eine neue Unterart der Maurischen Landschildkröte aus dem Sousstal (Südwest-Marokko). *Salamandra* **36**, 209-222.
- Pieh, A. (2001b). Eine Resolution gegen unverantwortliche Auswilderung von Schildkröten. *Radiata* **10**, 18.
- Puerto, G., Da Graça Salomão, M., Theakston, R. D. G., Thorpe, R. S., Warrel, D. A. & Wüster, W. (2001). Combining mitochondrial DNA sequences and morphological data to infer species boundaries: phylogeography of lanceheaded pitvipers in the Brazilian Atlantic forest, and the status of *Bothrops pradoi* (Squamata: Serpentes: Viperidae). *Journal of Evolutionary Biology* **14**, 527-538.
- Strauch, A. (1862). Chelonologische Studien mit besonderer Beziehung auf die Schildkrötensammlung der kaiserlichen Akademie für Wissenschaften zu St. Petersburg. *Mémoires de l'Académie Impériale des Sciences de St.-Petersbourg, VII^e Série* **5(7)**, 1-196 + 1 pl.
- Van der Kuy, A. C., Ballasina, D. L. Ph., Dekker, J. T., Maas, J., Willemsen, R. E. & Goudsmit, J. (2002). Phylogenetic relationships among the species of the genus *Testudo* [sic] (Testudines: Testudinidae) inferred from mitochondrial 12S rRNA gene sequences. *Molecular Phylogenetics and Evolution* **22**, 174-183.
- Weissinger, H. (1987). *Testudo graeca anamurensis* ssp. nov. aus Kleinasien. *ÖGH-Nachrichten* **10/11**, 14-18.

Accepted: 22.10.03