Range extension and species confirmation of *Rhyneptesicus nasutus* (*Sind Serotine Bat*) (Mammalia:Chiroptera) from Bajaur Agency, FATA, Pakistan

Muhammad Idnan1,4 · Sajid Mansoor2 · Muhammad Babar Khawar4 · Arshad Javid1 · Ali Hussain1 · Muhammad Imran3 · Arif Ullah3

Received: 27 August 2021 / Accepted: 18 November 2021 / Published online: 4 January 2022

© The Author(s), under exclusive licence to Springer Nature B.V. 2021

Abstract

Background The lack of morphological differentiation among chiropteran species and cryptic speciation impedes species identification. DNA-based approaches help species identification and contribute to the discovery of additional species. *Rhyneptesicus nasutus* (*Sind Serotine Bat*) is a rare and poorly studied species in Pakistan.

Methods This study explores the range extension of Sind Bat within the territorial limits of Pakistan from Sind and Baluchistan to Federally Administered Areas of Pakistan. No molecular record exists for the species in Pakistan. In the present study, we for the first time confirm species identification of *Rhyneptesicus nasutus* from Pakistan using a genetic marker (cytochrome b) along with morphometric analysis. A neighbor-joining tree based on Kimura-2 parameters was created to infer phylogenetic relationships. We sequenced the cytochrome b gene segment and conducted a phylogenetic analysis with previously published data from other countries.

Results Sequences from Pakistan formed a clade with Iranian *Rhyneptesicus nasutus* specimens suggesting a common ancestry. Various morphometric parameters (mean values) were measured, including Head and Body length (44.3 mm), Tail length (43.4 mm), Hindfoot length (8.3 mm), Forearm length (35.7 mm), and Ear length 36 mm while 5th Metacarpal Length, 4th Metacarpal Length, and 3rd Metacarpal Lengths were 33.2 mm, 34.7 mm, and 35.3 mm. Approaches based on DNA barcoding reveal a high diversity of bat species in the study area.

Conclusion The data will enable researchers to build an improved evolutionary framework of the Serotine Bats from this region and subsequently reconstruct a detailed evolutionary history of the genus. Further research is required to test other molecular markers to support the findings of the current study in Pakistan.

Keywords Chiroptera · Cytochrome b · Phylogenetic analysis · Mitochondrial · Eptesicus

Introduction

More than 8 million different animal species inhabit our planet of which only a few, about 1.5 million have been characterized [1]. Bats comprise roughly 20% of the mammalian diversity with around 1400 species discovered worldwide [2]. Bats being the 2nd most diverse group of mammals are essential to the ecological health of any ecosystem, by playing a vital role not only in several predator–prey relationships but also in the dispersal of seeds, plant pollination, and nutrients distribution/recycling [3].

Moreover, bats have a great impact on humans as well where bats are involved with insects and pest management. Bats feed on a number of insects that damage crops and which are serious forest pests. An analysis from a study of North
American insectivore bats highlighted that a loss of bats in that region leads to an annual agricultural loss of more than $3.7 billion. Bats are hunted for their bushmeat and medicine, are praised for their aesthetics (bat watching tourism), and are studied for educational and research purposes. However, it is of note that few species are themselves pests, impacting livestock and agriculture sector, damage buildings, and are potential reservoirs of many infectious agents [4].

Bats are extremely diverse, however, due to the cryptic speciation, this component of biodiversity is usually neglected. Furthermore, the exact characterization of any species is imperative for conservative efforts to conserve their vital ecological role [5]. Recent advancements in better interpretation of molecular biology data (superior phylogenetic reconstructions) have paved the way for the identification of these cryptic species. Moreover, combining these techniques with modern approaches that explore acoustic variables has vastly expanded the number of recognized bat species [6, 7].

*Rhyneptesicus nasutus* is present in Saudi Arabia, the Islamic Republic of Iran, Afghanistan, Iraq, Oman, Yemen, and Pakistan [8]. Interestingly, this species has probably never been abundant throughout its relatively restricted geographical range [9]. Furthermore, the species is rare and locally distributed in Pakistan where it has been reported from Kharan and Rajbar—Baluchistan, and Shikarpur—Sindh [10]. *R. nasutus* is known as the ‘Sind Serotine’ bat and its status is designated as “Least Concern” by IUCN [11].

Modern molecular techniques also suggest that in Southeast Asia the number of bat species is twice the number of currently recognized species. In areas of high endemism and hotspots of biodiversity, the cryptic species are more prevalent as such areas are considered to have a high potential of speciation [12]. Other than the cytochrome *b* gene, 16S rRNA and 12S rRNA could also be used for species identification, but the cytochrome *b* gene has been used for the last two decades for animal identification and differentiation among closely related taxa. Cytochrome *b* gene shows a high polymorphism which benefits species identification [13, 14].

Hence, the current study was designed for species identification, genetic differentiation, and phylogenetic analysis of *Rhyneptesicus nasutus* from Pakistan by using the mtDNA cytochrome *b* region from chiropteran taxon, Bajaur Agency, Federally Administered Areas (FATA) region of Pakistan.

**Materials and methods**

**Sampling**

The bat samples were captured from the FATA region, 32.6675° N, 69.8597° E, comprising a total area of 27,220 km² of Pakistan (Fig. 3). The roost sites of the bat were found in cervices and holes in buildings and caves. The information about the roosts of the bats was also collected from the nomads. The mist nets of different categories and different lengths (5 m, 8 m, and 11 m) were used for bats collections. The mist nets were applied mostly before the time of the evening. The nets were applied on water bodies and the narrow ways where the bats were more in number. The sampling was extended from June 2016 to August 2018. During the time frame of sampling, all the potential roosting sites were searched thoroughly to collect the sample.

**Sample preservation and measurements**

During sampling 215 bats samples belonging to different genera (*genus Pipistrellus*, *genus Scotophilus*, *genus Rhinopoma*, *genus Rousettus*, *genus Myotis*, and *genus Rhinophyllus*) were captured. Samples belonging to *Rhyneptesicus nasutus* species were collected. Various morphometric measurements were noted (Fig. 1). Some samples (n = 2) were preserved in 70% ethanol for molecular analysis. Bats were euthanized by placing a bat in an airtight zipper bag followed by the placement of an Isoflurane (3–5 ml) soaked cotton ball in the bag. This was closed for 40 min and it was ensured that the bat was not alive. The comparative observational analyses were performed with Bates and Harrison [9, 15].

**DNA extraction and amplification of cytochrome *b* gene**

Genomic DNA was extracted from ethanol (70%) preserved specimens (wing tissue i.e., 10 μg) at Post Graduate Lab, Institute of Biochemistry and Biotechnology (IBBt), University of Veterinary and Animal Sciences, Lahore, Pakistan, by standard phenol–chloroform extraction method [16]. Fragments of mtDNA were amplified using a set of primers described by Kocher 1989 forward primer 5-CCATCCAAC ATTCAGCATGATGAAA-3 and reverse primer 3-CCC TCAGAATGATTTGTCTCA-5 [17].

For PCR reaction a volume of 25 μL was used as a reaction mixture under conditions: initial denaturation at 95 °C for 4 min, 30-s denaturation at 94 °C, annealing at 59 °C for 30 s, 72 °C for 30 s for extension, and 72 °C for 10 min for a final extension. PCR products were identified using 1.5% agarose gel and positive samples were purified by ethanol for DNA sequencing using ABI PRISM Genetic Analyzer 3130xl [18].

**Sequence alignment and analysis**

Chromas software was used to analyze Cytochrome *b* gene sequences [19], BLASTn software available on NCBI was
used to align the sequences of cytochrome b for species confirmation by molecular analysis. Single Nucleotide Polymorphisms (SNPs) were detected from aligned sequences and consensus sequences, and haplotypes were constructed. MEGA X software was used by the Neighbor-joining method with Bootstrap values of 500 pseudoreplication [20]. Newly obtained DNA sequences were submitted to GenBank for Accession numbers MT674673 and MW842644. For phylogenetic reconstruction, Genbank Accession Numbers of *Rhynopecticus* species belonging to Iran were retrieved (FJ841981, FJ841980, EU786840, and EU786839), from Oman (KF019042, KF019043, KF019044, KF019041, and KF019040), from Yemen (KF019057, KF019056), from Germany (AF376836), from China (MG570068, EU786841), from Laos (EU786849) and from Patagonia (MK429705, MK429702, and MK429700). While a vampire bat species *Desmodus rotundus* (KU938397) was used as an outgroup in this phylogenetic reconstruction.

**Results**

**Molecular sequences**

The main objective of the study was to explore the bat diversity in the study region of FATA, Pakistan, and use the cytochrome b gene as a marker for species confirmation. Bats have cryptic morphological characters so taxonomic identification by morphology may be difficult. A small fragment of wing tissue yielded enough DNA for molecular study. PCR amplification of cytochrome b gene by specific primers produced a single amplicon with
1% agarose gel. These PCR products of *R. nasutus* from Bajaur Agency, FATA, Pakistan, were sequenced. These sequences were submitted to NCBI GenBank for Accession numbers MT674673 and MW842644. On NCBI, the query sequences were aligned and compared with previously published sequences of *Rhyneptesicus* species by BLASTn.

During the study, DNA sequences of chiropteran species representing *Rhyneptesicus* (formally known as *Eptesicus*) genera and the Vespertilionidae family were obtained. These DNA sequences have shown reliable and clear species identifications. Recently, DNA barcoding studies of Asian bats have been carried out and sequences of related species were available at NCBI. Closely related DNA sequences of cytochrome b were retrieved from public databases in blast searches. The neighbor-joining tree based on Kimura 2-parameter distance is shown in Fig. 2, where the query sequences are making a clade with Iranian *Rhyneptesicus* species, while the species from other regions like Oman, Yemen, China, Germany and Patagonia are not as close to *Rhyneptesicus* species reported from Pakistan. It suggests that these *Rhyneptesicus* species have evolved independently from the Iranian and Pakistani species.

The sequence results of the query sample were run in BLASTn, the percentage identity [of both new sequences?] was 95.1% with a sequence of *R. nasutus* (FJ841981) from Dehbarez, Hormozgan, Iran. Gene sequences of specimens of *Rhyneptesicus nasutus* and other species were retrieved from GenBank, and were used for phylogenetic analysis. MEGA X was used for phylogenetic analysis by the Neighbor-joining method with Bootstrap values of 500 pseudoreplications. A sequence of a common vampire bat (*Desmodus rotundus*) was used as an outgroup (Fig. 2).

Phylogenetic divergence among *Rhyneptesicus* species from different regions of the world is shown in Fig. 3. The genetic divergence (p distance) among various *Rhyneptesicus* species reported from various regions of the world shows a minimum value of 0.000–0.087. The p distance value among the Pakistani *Rhyneptesicus* species and others published from various parts of the world showed...
genetic differentiation among them suggesting that they have evolved independently from one another.

**Morphology**

*Rhyneptesicus* have a relatively long tail which is roughly equal to the head and body length. Forearm length averages 36.1 mm (35.4–36.9 mm). The muzzle is broad and flat with prominent nearly naked pararhinal glandular swellings. The ears are small with narrowly rounded tips: the tragus of each is well developed. About half the height of the pinna. The pelage is buffy brown on the dorsal surface and paler on the ventral surface. The hair tips and bases are uniformly colored. The interfemoral and wing membranes ears and naked areas of the face are mid-brown, distinctly darker than the pelage (Fig. 1).

The skull is smaller than that of *E. bottae* with an average condylo-canine length of 12.0 mm (11.7–12.2 mm). The braincase is relatively small and flattened in lateral view as compared to that of *Eptesicus bottae*. The sagittal crest is absent, and the lambdoid crests are low but distinct. The slightly convex supraoccipital forms the most posterior part of the skull. The postorbital constriction is narrow, and the supraorbital ridges are well developed. The zygomatic arches are delicate and without dorsal projections: they are widely flared anteriorly and posteriorly. The palate is short and broad. The tympanic bullae are relatively large and exceed the breadth of the basioccipital. The coronoid process of each half mandible does not greatly exceed the condyle in height: in consequence the posterior border of the coronoid declines more gently than that of *Eptesicus bottae*.

Upper tooth row length (c-m3) averages 4.6 mm (4.4–4.8 mm). The first upper incisor (i2) is without a secondary cusp. The second incisor (i3) is relatively well developed, attaining half the height of i2. The upper premolar (pm4) is relatively small; its crown area slightly exceeds half that of m1; in *Eptesicus bottae*, it is more than three quarters, m3 is about half the crown area of m2 and with three well-developed commissures. The first lower premolar (pm2) is relatively small; it is crowded between the canine and the second premolar (pm4), m1 and m2 are roughly equal in size, m3 has the talonid only slightly reduced. In Baluchistan, it was collected at 1108 m (3600 feet) at the junction of Razhai & Sichk Rivers (BMNH). In Iran, it was reportedly common in the town of Ahwaz (at an altitude of 68 m: 220 feet) (BMNH). In Oman, it was found secreted in the walls of ruined buildings, isolated in semi-desert terrain [21]. In Afghanistan, three female specimens were collected at Lindberg’s Cave, 25 miles north of Jalalabad: a further 23 specimens of both sexes were collected at an altitude of 738 m (2400 feet) miles north of Jalalabad and a single female specimen from 862 m (2800 feet) at Laghman.

**Discussion**

**Molecular identification**

Bats are a very poorly studied group of mammals in Pakistan. In the current study, we studied bats of Bajaur Agency, FATA region of Pakistan. Molecular identification and phylogenetic analysis has not been used for bats in Pakistan, and this is the first attempt to do so. The study area is a hilly area which is not easily modified as compared to plain areas for construction activities that is why bats are surviving in these areas easily. Due to the lack of prior molecular data on the genus *Rhyneptesicus* in Pakistan, the data we collected allow us to draw conclusions about this genus.

This study describes the comparison of cytochrome b sequences for *Rhyneptesicus* species from Pakistan, Iran, Oman, Germany, Laos, Yemen, China, and Patagonia to access the genetic divergence and evolutionary analysis among these localities of the world. The distribution and divergence in different parts of the world suggest that *Rhyneptesicus* species have evolved and spread in various localities. The close phylogenetic relationship of Pakistani and Iranian specimens of *R. nasutus* suggests that they share a common ancestry as compared to the others reported from the world. No data of cytochrome b from the Afghanistan region is available so we can not infer any cladistic relationship of Pakistani *Rhyneptesicus* species with Afghanistan’s specimens.

Genetic markers like mtDNA and nuDNA describe a geographic and genetic relatedness for discontinuous distribution of the genus *Rhyneptesicus*. The taxonomic reconstruction of *R. n. nasutus* samples from Iran which are close to Pakistan validates the subspecies recognition [8] hence this study also confirms its presence by range extension and validation by molecular marker (cytochrome b gene). Current molecular investigations have placed *Eptesicus* in tribe Nectarinini, separating it from pipistrelles [22]. For phylogenetic reconstruction, Genbank Accession Numbers of *Rhyneptesicus* species belonging to Iran were retrieved (FJ841981, FJ841980, EU786840, and EU786839), from Oman (KF019042, KF019043, KF019044, KF019041, and KF019040), from Yemen (KF019057, KF019056), from Germany (AF376836), from China (MG570068, EU786841), from Laos (EU786849) and Patagonia (MK429705, MK429702, and MK429700).

While a vampire bat species *Desmodus rutundus* (KU938397) was used as an outgroup in this phylogenetic reconstruction. The genetic divergence of these *Rhyneptesicus* species is given in Fig. 3.
Faunistics

The previous records of Rhynepetesicus nasutus in Pakistan were reported from Shikarpur, Sind, Pakistan. According to Blanford (1888–1891) the type locality should be a little east of Rohri’ [23] but the recent record from Bajaur agency FATA, Pakistan highlights the range extension of Sindh Bat as this bat species has not been previously reported from this area. These records of R. nasutus from Bajaur Agency might represent migrants from Afghanistan or may be local residents in Bajaur. Here, we also highlight the difference in weather conditions of Bajaur Agency which is the hilly area while the Shikarpur, Sind, is a plain area where it was reported by Blanford, 1888–1891. It also suggests that a detailed study on the comparison of ecological aspects should be conducted for further conservation action plans of this species.

No complete record for bats in Pakistan is available right now. Currently, some people are involving in research work about taxonomic and phylogenetic studies of bats. In the future, hopefully, enough data will be available to cover the exact taxonomic positions of various bat species from Pakistan. Juste et al. reassigned this taxon to the genus Rhynepetesicus Bianchi, 1917 based on molecular phylogenetics [9]. Four subspecies—R. n. nasutus (Southwest Pakistan, Afghanistan, and Southeast Iran), R. n. matschiei (Southwest Arabia), R. n. pellucens (Iran and Iraq), and R. n. batinensis (Eastern Arabia including Oman and Saudi Arabia), are recognized [8, 24].

The distribution record of R. nasutus is wide and patchy. It has been reported from Arabian Peninsula to western South Asia, recorded from Oman, Saudi Arabia, Yemen, United Arab Emirates (UAE), Qatar, Kuwait, southeastern Iran, and southern Iraq. From South Asia, it has been reported from Afghanistan and Pakistan, but from the territorial boundary of Pakistan, it is just reported from Baluchistan and Sindh [9, 25, 26]. However, the new record for range extension of R. nasutus is reported from Bajaur Agency, FATA, Pakistan. The distributional record of R. nasutus in Baluchistan (Seistan) is more or less continuous while in Afghanistan (Jalalabad valley) its occurrence is 700–800 km away to the nearest record of central Pakistan [9]. Conversely, in the Middle East, the occurrence of Rhynepetesicus nasutus has been reported in a mosaic of isolated patches as compared to in a continuous belt [27, 28], this might be the case for its distributional range (Province Sindh and Baluchistan and then in the mountainous area of Bajaur Agency) in Pakistan where Bajaur distribution in central Pakistan represents another such patch; Bates [29] summarized three records from central Baluchistan (Kharan, Rajbar, a junction of the Razhai, and Sichk rivers; [9, 30], one from northern Sindh (near Rohri; [23] and current study explores the identification and the range extension to northern Pakistan. Where an extensive survey should be conducted to explore more roost sites and population dynamics of Rhynepetesicus.

It is summarized that the effects of climate change on the range extension of Rhynepetesicus may be primarily determined by the weather consequences on the habitat requirements and physiological tolerances of the species under study. Here, in the case of R. nasutus evolution in ecologically different environment of Bajaur Agency, Pakistan may be due to variable environmental conditions as compared to its prior occurrence in other regions of the country, i.e., Baluchistan and Sind province. The data obtained during this study is very significant as no record about phylogenetic analysis of bats is available in Pakistan.

Bat populations appear to be declining presumably in response to human-induced environmental stresses like habitat destruction and fragmentation, disturbance to caves, depletion of food resources, overhunting for bushmeat and persecution, increased use of pesticides, infectious disease, and wind energy turbines. As bats are among the most overlooked despite their economical and ecological importance, their conservation is mandatory. An extensive study is recommended to explore the distribution range and highlight the significance of bats throughout the territorial limits of Pakistan.

Conclusion

Sindh Bat has a limited geographic range. The distributional range of this species is not thoroughly explored within the territorial limits of Pakistan. In Pakistan, it has only been reported from Sindh and Baluchistan. The present record of range extension of Rhynepetesicus nasutus is reported for the first time from the FATA region of Pakistan based on both morphological characteristic and genetic analyses (cytochrome b). An extensive study with other molecular markers is recommended throughout the country so that the findings of the current study may be strengthened and augmented.

Funding No funding was received from anybody. All the expenditures for research work were supported by the authors.

Declarations

Conflict of interest The authors declare no conflict of interest with anyone.

Ethical approval The author(s) declares that the animals used in this research work were handled with ethical standards for animal care and use in research approved by Society for Prevention of Cruelty to Animals (SPCA, University of Veterinary and Animal Sciences, Lahore, Pakistan).
References
1. Mora C, Tittensor DP, Adl S, Simpson AGB, Worm B (2011) How many species are there on Earth and in the ocean? PLoS Biol 9:e1001127
2. Burgin CJ et al (2018) How many species of mammals are there? J Mammal 99(1):1–14
3. Boyles JG et al (2011) Economic importance of bats in agriculture. Science 332(6025):41–42
4. Garcia-Herrera LV, Ramírez-Fráncel LA, Guevara G, Reinoso-Flórez G, Sánchez-Hernández A, Lim BK, Losada-Prado S (2021) Foraging strategies, craniodental traits, and interaction in the bite force of Neotropical frugivorous bats (Phyllostomidae: Stenodermatinae). Ecol Evol 11:13756–13772
5. Bickford D et al (2007) Cryptic species as a window on diversity and conservation. Trends Ecol Evol 22(3):148–155
6. Wilson DE, Reeder DM (2005) Mammal species of the world: a taxonomic and geographic reference. JHU Press, Baltimore
7. Theodoridis S, Fordham DA, Brown SC, Li S, Rahbek C, Nogués-Bravo D (2020) Evolutionary history and past climate change shape the distribution of genetic diversity in terrestrial mammals. Nat Commun 11(1):1–11
8. Clare EL (2011) Cryptic species? Patterns of maternal and paternal gene flow in eight Neotropical bats. PLoS ONE 6(7):e21460
9. Juste J, Benda P, Garcia-Mudarra JL, Ibanez C (2013) Phylogeny and systematics of Old World serotine bats (genus Epotesicus, Vespertilionidae, Chiroptera): an integrative approach. Zoolog Scr 42(5):441–457
10. Bates P, Harrison D (1998) Bats of the Indian subcontinent. Biodivers Conserv 7(10):1383–1386
11. IUCN (2021) The IUCN red list of threatened species. Version 2021-2. Available at https://www.iucnredlist.org
12. Francis CM et al (2010) The role of DNA barcodes in understanding and conservation of mammal diversity in Southeast Asia. PLoS ONE 5(9):e12575
13. Linacre A, Lee JCI (2016) Species determination: the role and use of the cytochrome b gene. Forensic DNA typing protocols. Humana Press, New York, pp 287–296
14. Vindigni G, Pulvirenti A, Alaimo S, Monaco C, Spina D, Peri I (2021) Bioinformatics approach to mitigate mislabeling in EU Seafood market and protect consumer health. Int J Environ Res Public Health 18(14):7497
15. Roberts TJ (1977) The mammals of Pakistan. E. Benn, London
16. Wang TY, Wang L, Zhang JH, Dong WH (2011) A simplified universal genomic DNA extraction protocol suitable for PCR. Genet Mol Res 10(1):519–525
17. Kocher TD et al (1989) Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. Proc Natl Acad Sci 86(16):6196–6200
18. Sanger F, Nicklen S, Coulson AR (1977) DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci 74(12):5463–5467
19. Pty T Ltd (2018) Chromas lite software
20. Kumar S et al (2018) MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol 35(6):1547–1549
21. Harrison D, Bates P (1991) The mammals of Arabia. Harrison Zoological Museum, Kent
22. Hoofer SR, Van Den Bussche RA, Horáček I (2006) Generic status of the American pipistrelles (Vespertilionidae) with description of a new genus. J Mammal 87(5):981–992
23. Blanford WT (1898) The Fauna of British India: including Ceylon and Burma, vol 4. Taylor & Francis, Abingdon-on-Thames
24. Comelis MT, Bueno LM, Góes RM, Taboga SR, Morielle-Versute E (2018) Morphological and histological characters of penile organization in eleven species of molossid bats. Zoology 127:70–83
25. Zeale MR, Bennitt E, Newson SE, Packman C, Browne WJ, Harris S, Stone E (2016) Mitigating the impact of bats in historic churches: the response of Natterer’s bats Myotis nattereri to artificial roosts and deterrence. PLoS ONE 11(1):e0146782
26. Srinivasulu C et al (2019) Integrated approaches to identifying cryptic bat species in areas of high endemism: the case of Rhinolophus andamanensis in the Andaman Islands. PLoS ONE 14(10):e0213562
27. Benda P et al (2010) Noteworthy records of bats from Yemen with description of a new species from Socotra. Hystrix Italian J Mammal 22(1):23–56
28. Dool SE, Puechmaille SJ, Foley NM, Allegrini B, Bastian A, Mutumi GL, Jacobs DS (2016) Nuclear introns outperform mitochondrial DNA in inter-specific phylogenetic reconstruction: lessons from horseshoe bats (Rhinolophidae: Chiroptera). Mol Phylogenet Evol 97:196–212
29. Bates PJ (1997) Bats of the Indian Subcontinent: Harrison Zoological Museum publication. Sevenoaks, Kent, United Kingdom
30. Roberts T (1997) The mammals of Pakistan (revised ed). Oxford University Press, Karachi

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.