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On two shells of trionychid turtles in the collection of the "Zoologische Staatssammlung München" with remarks about morphological differences between *Chitra* GRAY, 1844 and *Pelochelys* GRAY, 1864
(Testudines: Trionychidae)

Über zwei Schalen von Weichschildkröten in der Zoologische Staatssammlung München, mit Bemerkungen zu morphologischen Unterschieden zwischen *Chitra* GRAY, 1844 und *Pelochelys* GRAY, 1864
(Testudines: Trionychidae)

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KURZFASSUNG

Belegmaterial von Riesen-Weichschildkröten der Gattungen *Chitra* und *Pelochelys* ist in Museumssammlun - gen selten. Zwei Weichschildkrötenschalen aus der Zoologischen Staatssammlung München, die unter dem Namen *Pelochelys cantorii* GRAY, 1864 inventarisiert sind, wurden deshalb und wegen einer bemerkenswerten Fundortangabe mit morphologischen und genetischen Methoden in Hinblick auf ihre Artbestimmung überprüft. Eine der beiden Schalen aus der Sammlung SCHLAGINTWEIT stammt aus Sindh, Pakistan, und wäre ein Erstnachweis von *P. cantorii* für das Industal. Die Analyse ergibt, daß beide Schalen von *Chitra indica* (GRAY, 1831) stammen. In einer Tabelle werden überdies morphologische Merkmale des knöchernen Panzers zur Unterscheidung von *Pelochelys* und *Chitra* zusammengestellt.

ABSTRACT

Preserved voucher specimens of giant trionychid turtles of the genera *Chitra* and *Pelochelys* are rare in scientific museum collections. Therefore, and because of the remarkable record locality, two shells in the collection of the "Zoologische Staatssammlung" in Munich, Germany, attributed to *Pelochely cantorii* GRAY, 1864 were examined by morphological and genetic methods to verify their taxonomic allocation. One of these shells, originating from the SCHLAGINTWEIT collection, was collected in Sindh, Pakistan, and would be the first record of *P. cantorii* from the Indus river system. The analysis shows that both shells are to be assigned to *Chitra indica* (GRAY, 1831). Furthermore, a table of shell characters is provided to distinguish *Chitra* and *Pelochelys*.

KEY WORDS

Reptilia: Testudines: Trionychidae: *Chitra*, *Chitra indica*, *Pelochelys*, *Pelochelys cantorii*, bony carapace, plastron, morphology, DNA sequence analysis, identification, distribution, Indus Valley, Pakistan

INTRODUCTION

The giant trionychid turtles of the genera *Chitra* GRAY, 1844 (narrow-headed softshell turtles) and *Pelochelys* GRAY, 1864 (Asian giant softshell turtles), constituting (unluckily together with *Amyda*) the tribe Chitrini sensu MEYLAN (1987), are all little studied, and their systematics and distribution are extremely little known. In 2002, MCCORD & PRITCHARD added considerably to the knowledge of the genus *Chitra* and also provided a review. Until then, two species of each genus, *Chitra* and *Pelochelys*, were recognized: *Chitra indica* (GRAY, 1831), *Chitra chitra* NUTAPHAND, 1986, *Pelochelys bibroni* (OWEN, 1853) and *Pelochelys cantorii* GRAY, 1864. MCCORD & PRITCHARD (2002) described hereupon two new taxa of *Chitra*, *Chitra chitra javanensis* and *Chitra vandijki*. Moreover, they evaluated all relevant literature sources in their paper, which includes a bibliography. Due to the rarity of *Chitra* in the wild throughout their range, the description of these new forms became possible only by consideration of captive specimens, in addition to preserved material deposited in scientific collections. In the

same issue of the journal Hamadryad, WEBB (2002) described a new species of *Pelochelys*, *Pelochelys signifera,* to be distinguished from its congeners by juvenile coloration and pattern. However, the description of a new species based mainly on the coloration of a single juvenile (which represents the type specimen) appears questionable. It is evident that these circumstances reflect the general scarcity of giant trionychid material in museum collections.

Because of the rarity of preserved giant softshell turtles and reliable published records, there is insufficient knowledge about the distribution ranges of the various taxa. Interestingly, a trionychid disk in the collection of the "Zoologische Staatssammlung" in Munich, Germany, which originated from the Indus river, was attributed to *Pelochelys cantorii*, although *P. cantorii* was not recorded previously from the Indus river system. However, this species can reasonably be expected to occur/or have occurred there, at least in historical times. The reason for this assumption is the recently documented occurrence of *Batagur baska* (GRAY, 1830) in the Indus Valley by PRASCHAG et al. (2008). The voucher upon which this record is based, is a stuffed male deposited in the Natural History Museum Vienna (NHMW 1841 - Indus Delta, Sindh, from the Munich museum, without date), which documents the presence of this species in the Indus Valley for the first time. As *Batagur*, *Chitra* and *Pelochelys* share large parts of their distribution (FRITZ & HAVAS 2007), it is plausible to assume that *P. cantorii* could occur in the Indus valley, too. Moreover, the *B. baska* specimen originates from the SCHLAGINTWEIT Collection, which is the same source from which the alleged *P. cantorii* shell deposited in Munich came. Additionally, GUIX (2001) reported on a huge specimen of a giant softshell turtle assigned to *Pelochelys bibroni* that washed ashore at the beach of Moçâmedes, Angola, southwestern Africa. This specimen is documented by a photograph kept at the Arquivo Fotografico Municipial de Lisboa, Portugal, and further corroborates that the knowledge of the distribution and possible marine migration of *Pelochelys* is most incomplete.

MATERIALS AND METHODS

Morphological analysis

We examined morphological and genetic features of the abovementioned shell [Shell 1] from the collection of the "Zoologische Staatssammlung München" (ZSM) to verify its taxonomic assignment. In addition, we analyzed another specimen from the same collection, also labeled *P. cantorii* [Shell 2]. For the purpose of comparison, we adapted our characterization mainly to the genus description of *Chitra* by MCCORD & PRITCHARD (2002). The shell of *Pelochelys* was detailed in SIEBENROCK (1902), SMITH (1931) and PRITCHARD (1979). In Table 1, we compiled the relevant characters reported in the literature along with those determined in the present investigation.

Materials examined: [Shell 1] ZSM 2514/0 labeled "*Pelochelys cantoris* GRAY, Indus-Delta, Sindh, Westl. Indien", SCHLAGINTWEIT leg. [without date]. [Shell 2] ZSM 54/0 labeled "*Pelochelys cantorii* GRAY", provenance unknown.

Genetic analysis

For the genetic analysis the following samples where taken for DNA extraction: from Shell 1 (ZSM 2514/0) a piece of bone of the cervical vertebra; from Shell 2 (ZSM 54/0) a piece of cartilage from the inner side of the carapace. DNA was extracted using the All-tissue DNA Kit (GEN-IAL®, Troisdorf, Germany). After grinding the material in a Mixer Mill MM 400 (Retsch®, Haan, Germany) extraction was performed according to the manufacturer's protocol for DNA preparation from bones, with a final elution in 100 µl buffer. A control extraction without tissue was done to test for contaminated reagents. PCR primers for partial regions of the *cytochrome b* gene were designed specifically based on sequences of *Chitra* and *Pelochelys* published in GenBank (see below): cyc-cytb1+ (5'-acctacgaaaatcccaccc-3'), cyc-cytb3- (5'-gataatcccggtgtttcagg-3'), pelo-cytb5- (5'-aactgatgagaatgctgttg-3'), chi-cytb6- (5'-gattgatgagaatgct-

Fig. 1: *Chitra indica*, ZSM 2514/0, Indus, Sindh, Coll. SCHLAGINTWEIT.
Bony disk, dorsal aspect, fourth discrete neural missing.

Abb. 1: *Chitra indica*, ZSM 2514/0, Indus, Sindh, Coll. SCHLAGINTWEIT.
Knöcherner Diskus von dorsal, das vierte Neurale fehlt.

Fig. 2: *Chitra indica*, ZSM 2514/0, Indus, Sindh, Coll. SCHLAGINTWEIT.
Bony disk, visceral aspect, fourth discrete neural missing.

Abb. 2: *Chitra indica*, ZSM 2514/0, Indus, Sindh, Coll. SCHLAGINTWEIT.
Knöcherner Diskus von visceral, das vierte Neurale fehlt.

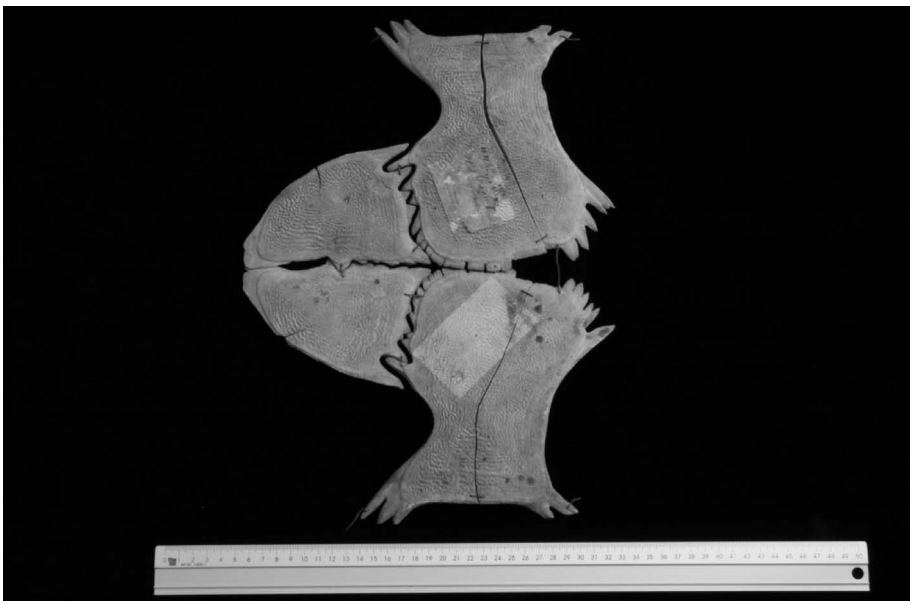

Fig. 3: *Chitra indica*, ZSM 2514/0, Indus, Sindh, Coll. SCHLAGINTWEIT.
Bony plastron in ventral aspect, preplastra and epiplaston missing.

Abb. 3: *Chitra indica*, ZSM 2514/0, Indus, Sindh, Coll. SCHLAGINTWEIT.
Knöcherner Plastron von ventral, Präplastra und Epiplastron fehlen.

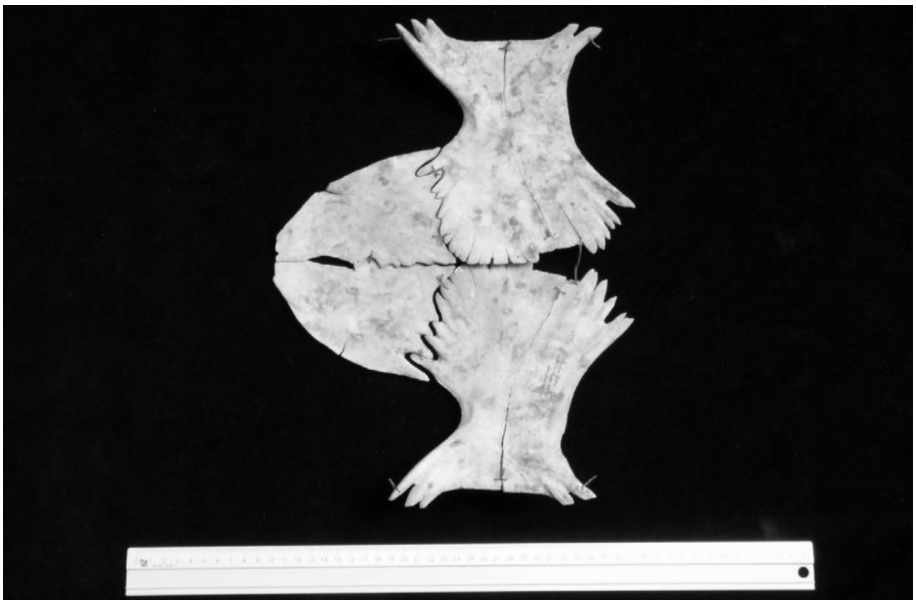

Fig. 4: *Chitra indica*, ZSM 2514/0, Indus, Sindh, Coll. SCHLAGINTWEIT.
Bony plastron in visceral aspect, preplastra and epiplaston missing.

Abb. 4: *Chitra indica*, ZSM 2514/0, Indus, Sindh, Coll. SCHLAGINTWEIT.
Knöcherner Plastron von visceral, Präplastra und Epiplastron fehlen.

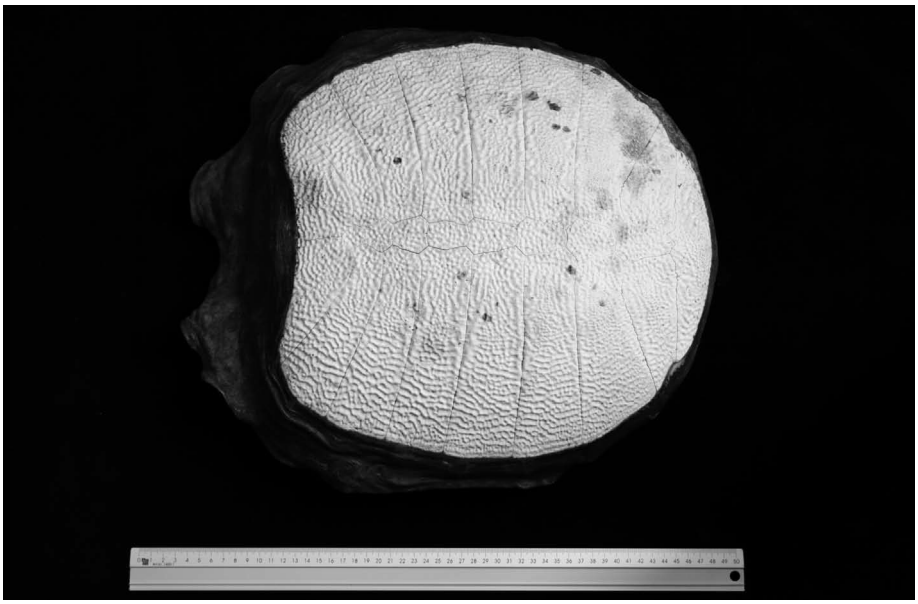

Fig. 5: *Chitra* sp., ZSM 54/0, of unknown origin, carapace, dorsal aspect.
Abb. 5: *Chitra* sp., ZSM 54/0, Herkunft unbekannt, Carapax von dorsal.

Fig. 6: *Chitra* sp., ZSM 54/0, of unknown origin, carapace, visceral aspect.
Abb. 6: *Chitra* sp., ZSM 54/0, Herkunft unbekannt, Carapax von viszeral.

Fig. 7: *Chitra* sp., ZSM 54/0, of unknown origin, plastron, ventral aspect.
Abb. 7: *Chitra* sp., ZSM 54/0, Herkunft unbekannt, Plastron von ventral.

Fig. 8: *Chitra* sp., ZSM 54/0, of unknown origin, plastron, visceral aspect.
Abb. 8: *Chitra* sp., ZSM 54/0, Herkunft unbekannt, Plastron von viszeral.

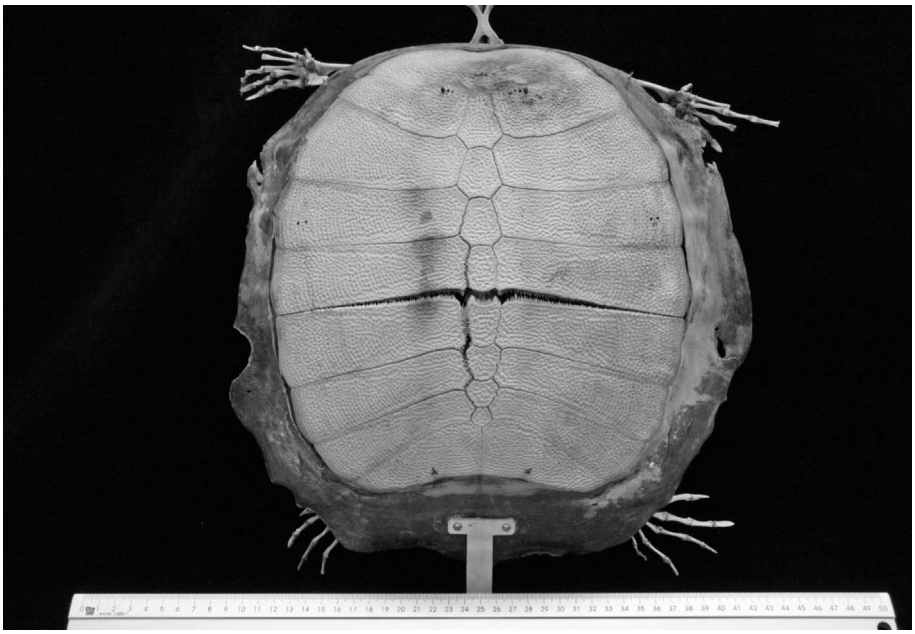

Fig. 9: *Pelochelys cantorii* NHMW 1857, Ganges, don. STEINDACHNER 1896, carapace, dorsal aspect.
Abb. 9: *Pelochelys cantorii* NHMW 1857, Ganges, don. STEINDACHNER 1896, Carapax von dorsal.

Fig. 10: *Pelochelys cantorii* NHMW 1857, Ganges, don. STEINDACHNER 1896, plastron, visceral aspect.
Abb. 10: *Pelochelys cantorii* NHMW 1857, Ganges, don. STEINDACHNER 1896, Plastron von viszeral.

gttg-3'). In the PCR reactions, primer cyc-cytb1+ was combined with one of the other three primers to obtain a 191 bp-fragment (combined with pelo-cytb5- or chi-cytb6-) or a 347 bp-fragment (combined with cyc-cytb3-), respectively. PCR (35 reaction cycles) was performed on a Master gradient thermocycler (Eppendorf) in 25 µl with 0.2 units TopTaq DNA Polymerase (QIAGEN), 1 µM of each primer, and 0.2 mM of each dNTP (QUIAGEN). Control reactions to detect contaminations were carried out with: (i) control 'extractions' (without sample) instead of the template, and (ii) with distilled water instead of the template. PCR products were extracted from agarose gels using the QIAquick Gel Extraction Kit (QIAGEN) and cloned (TOPOTA Cloning® Kit; Invitrogen). Sequencing of both strands was performed by AGOWA (Berlin, Germany). Editing and alignment of sequences were performed using the BioEdit software package version 5.0.9 (HALL 1999). For sequence comparison we used sequences of *C. indica* (GenBank accession number AY259561), *P. cantorii* (AY259560), and *P. bibroni* (AY259559) published by ENGSTROM et al. (2004).

RESULTS AND DISCUSSION

Morphological analysis

Shell 1 (ZSM 2514/0) - Bony disk (Figs. 1, 2): straight carapace length (CL) 35.5 cm, straight carapace width (CW) 32.0 cm (widest between the 3rd and 4th pleurals). Surface ornamentation vermiform, neurals (N) 1–4 hexagonal with shortest sides directed posteriorly (N4 missing), N5 oval shaped, N6–7 hexagonal with shortest sides directed anteriorly, N8 pentagonal. Pleurals number eight well developed, in full contact along the midline. Plastral elements consisting of the hyo-hypoplastra and xiphiplasta only (epiplastra and entoplastron missing) (Figs. 3, 4). Median portion of hyo-hypoplastral suture deviates anteriorly from a hypothetical straight transversal hyo-hypoplastral line in forming a sharp angle (24°/26° left/right). *Processus medialis anterior* (sensu SIEBENROCK 1902) of hyoplastron with three bi- and trifurcated prongs, the latero-anterior prong separated from the others; hypoplastron with six/five (left/right) broadened processes. Callosities cover almost the entire hyo-hypoplastra and xiphiplastra.

Shell 2 (ZSM 54/0) - Carapace bony elements complete (Figs. 5, 6): surface ornamentation vermiform, CL (including the dried skin) 42.5 cm, CW 34.0 cm (widest between the 3rd and 4th pleurals), same neural configuration as in ZSM 2514/0. Plastron (Figs. 7, 8): bony elements complete, entoplastron large boomerang-shaped and distinctive, anterior free portion of epiplastra almost as long as posterior part, supporting a front lobe of the plastron. *Processus medialis anterior* of hyoplastron with three bi- and trifurcated prongs, the latero-anterior prong not distinctly separated from the others, hypoplastron with five broadened, stubby processes. Median portion of hyo-hypoplastral suture deviates anteriorly from a hypothetical straight transversal hyo-hypoplastral line in forming a sharp angle (25°/28° left/right).

Comparing these morphological features to the descriptions reported in the literature (Table 1) reveals that the two specimens analyzed clearly match the morphological characterization of *Chitra* rather than that of *Pelochelys*.

Genetic analysis

Using various primer combinations we attempted to amplify *cytochrome b* sequences from the two samples. We obtained clear PCR products in three reactions: From specimen Shell 1 (ZSM 2514/0) the 191 bp fragment (cyc-cytb1+ / chi-cytb6-) and the 347 bp fragment (cyc-cytb1+ / cyc-cytb3-) were obtained. From specimen Shell 2 (ZSM 54/0) only the 191 bp fragment could be amplified (cyc-cytb1+ / pelo-cytb5-).

These sequences (without primer sequences the sections comprise 152 bp and 308 bp, respectively) are registered under the GenBank accession numbers HQ853298 (ZSM 2514/0) and HQ 853297 (ZSM 54/0). The sequences of Shell 1 and Shell 2 differ in

Table 1: Shell characters distinguishing *Chitra* GRAY, 1844 from *Pelochelys* GRAY, 1864. Note that only discrete neurals are counted. The first neural element may be a fused element corresponding with neurals 1 and 2 of other trionychid genera such as Cyclanorbinae and *Aspideretes* sensu MEYLAN (1987). MEYLAN (1987) considered them as separated. Numbers in parentheses are rarely encountered conditions.

Tab. 1: Schalenmerkmale zur Unterscheidung von *Chitra* GRAY, 1844 und *Pelochelys* GRAY, 1864. Es werden nur diskrete Neuralia gezählt. Das kranialste Neuralier-Element kann ein verschmolzenes Element sein, das den Neuraliern 1 und 2 bei anderen Trionychiden-Gattungen wie Cyclanorbinae und *Aspideretes* sensu MEYLAN (1987) entspricht. MEYLAN (1987) betrachtete sie als getrennt. Zahlen in Klammern bezeichnen selten auftretende Zustände.

	Character (feature) Merkmal	*Chitra*	*Pelochelys*
Carapace Carapax	Number of discrete neurals Anzahl diskreter Neuralia	8	(6, 7) 8
	Location of reversal in neural orientation Umkehrstelle in der Ausrichtung der Neuralia	5/6 (5)	(4/5, 5) 5/6 (6)
	Position of anterior edge of first body vertebra relative to nuchal bone Lage des Vorderrandes des ersten Rumpfwirbels in bezug zum Nuchale	anterior edge of nuchal Vorderrand des Nuchale	middle of nuchal Mitte des Nuchale
	Surface ornamentation Oberflächenskulpturierung	vermiform wurmförmig	pitted grubig
Plastron	Inner anterior spurs of hyoplastra (*Processus medialis anterior*) Innere vordere Hyoplastralsporne (*Processus medialis anterior*)	only 3 spines (occasionally bi- and trifurcated) nur 3 Dorne (gelegentlich zwei- und dreifach gegabelt)	quite uniformly serrated, with 6 to 8 spines recht gleichmäßig gesägt, mit 6 bis 8 Dornen
	Protuberances at posteromesial edge of hypoplastra Vorsprünge auf dem posteromesialen Rand der Hypoplastra	stubby and spaced out (with 3 to 4 jags) gedrungen, breit, spatelförmig (mit 3 bis 4 Kerben)	spines, occasionally bifurcated Dorne, gelegentlich gegabelt
	Anterior process of epiplastron Vorderer Epiplastralfortsatz	short, but developed, producing a front lobe of plastron kurz, gut entwickelt, einen Plastralvorderlappen bildend	very short, anterior border of plastron rounded without lobe sehr kurz, Plastron-Vorderrand rund, ohne Lappen
	Angle between hypothetical straight transversal hyo-hypoplastral line and median portion of hyo-hypoplasral suture Winkel zwischen einer hypothetischen geraden transversalen Hyo-Hypoplastrallinie und dem medianen Abschnitt der Hyo-Hypoplastralnaht	distinct, sharp angle, 25°-28° deutlich, Winkel spitz, 25°-28°	suture waved (rolling), angle very sharp, 12°-15° Naht wellig, Winkel sehr spitz, 12°-15°

one position within the 152 bp section and are almost identical with the sequences of *Ch. indica*. In the 308 bp section (Shell 1) the p distance is 0.3%. As the distances between the GenBank sequences of *Pelochelys* (*P. cantorii*, *P. bibroni*) and *Ch. indica* are 14.0% and 12.0%, respectively, it is quite clear that the specimens analyzed do not represent *Pelochelys* but *Chitra*. The high sequence similarity is in accordance with the assumption that Shell 1 (ZSM 2514/0) and Shell 2 (ZSM 54/0) belong to *Ch. indica*. Sequence divergence between this species and the related species *Chitra vandijki* and *Chitra chitra* (comparing the 308 bp section) are 6.2% and 5.2%, respectively. It remains to be investigated whether intraspecific differentiation within *Ch. indica* reveals a phylogeographic structure. This would require a comprehensive study of samples covering the whole distribution range.

Conclusions

In summary, morphological and genetic data agree that both shells, ZSM 2514/0 and ZSM 54/0, are to be assigned to the genus *Chitra*, and more specifically to *Ch. indica*. The disk from the Province of Sindh (Pakistan) is the first voucher of the species from that region, at least from the 19th Century. The occurrence of narrow-headed softshell turtles in the Indus Valley is current state of knowledge (DAS & SINGH 2009).

Adult turtles of the genera *Chitra* and *Pelochelys* are highly sought after as human food and there is a good chance to find bony disks and plastra in middens where these turtles occur (comp. MOLL 1987; SINGH et al. 2010). Table 1 provides an overview of the bony shell morphological differences between *Chitra* and *Pelochelys* in addition to the description of the two shells studied. This compilation should allow identification of adult specimens' bony shells. It is based on the authors' observations (GEMEL unpublished) and those of MEYLAN (1987) and MCCORD & PRITCHARD (2002) as well as data by SIEBENROCK (1902), which were not included in MCCORD & PRITCHARD (2002). A mounted skeleton of *Pelochelys cantorii* (NHMW 1857, Ganges, don. STEINDACHNER 1896) was used for comparison (Figs. 9, 10).

The following characteristics distinguish the bony disks of *Chitra* and *Pelochelys* from the disks of other trionychid turtles of the Indian subcontinent such as *Lissemys*, *Nilssonia* sens. lat., *Amyda*, *Pelodiscus*, *Palea* and *Dogania*: Shell large, bony disk reaching about 80 cm in *Chitra* and 60 cm in *Pelochelys* (SMITH 1931), flat, about as long as wide, in old specimens (CL > 30 cm). Broad callosities on hyo-hypoplastra and xiphiplastra, but no callosities on either the entoplastron and the epiplastra. Triangular anterior median spur-like process of xiphiplastra extending between hypoplastra. Permanent mid-plastral fontanelles present, one of them small, oval-shaped, along the midline between xiphiplastra.

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