ZOOLOGICAL SCIENCE **22**: 353–362 (2005)

Phylogenetic Relationships of the Tribe Paini (Amphibia, Anura, Ranidae) Based on Partial Sequences of Mitochondrial 12s and 16s rRNA Genes

Jianping Jiang[1], Alain Dubois[2*], Annemarie Ohler[2], Annie Tillier[3],
Xiaohong Chen[4], Feng Xie[1] and Matthias Stöck[5†]

[1]*Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu 610041, China*
[2]*Reptiles et Amphibiens, UMS 2700, Département Systématique & Evolution,*
Muséum National d'Histoire Naturelle, 25 rue Cuvier, 75005 Paris, France
[3]*Service de Systématique Moléculaire, CNRS IFR 101, Muséum National d'Histoire*
Naturelle, 43 rue Cuvier, 75005 Paris, France
[4]*School of Life Sciences, Henan Normal University, Xinxiang 453002, China*
[5]*Staatliche Naturhistorische Sammlungen Dresden, Museum für Tierkunde, A.-B.-Meyer-Bau,*
Königsbrücker Landstr. 159, 01109 Dresden, Germany

ABSTRACT—Partial sequences of mitochondrial 12S and 16S rRNA genes from 19 Asian frog species of the tribe Paini (Ranidae, Dicroglossinae) allowed a first molecular study of the phylogenetic relationships of this tribe. This analysis confirmed that this tribe is a monophyletic group, but suggested relationships did not agree with previous generic classification of this clade based on morphology. Two major clades were recognized within the Paini. For one of them, the generic name *Quasipaa* is available. Phylogenetic relationships within the other group are not yet fully clarified and need further study.

Key words: Amphibia, Ranidae, Paini, phylogeny, mitochondrial DNA, Asia

INTRODUCTION

The frog family Ranidae, as traditionally understood (Duellman and Trueb, 1985), shows a very wide (almost cosmopolitan) geographical distribution and of a large number of included taxa (species, species-groups, subgenera and genera), so that for the time being the phylogenetic and taxonomic relationships within this family are still largely conjectural and controversial. In a recent taxonomic proposal (Dubois, 2003), eleven subfamilies were recognized within the Ranidae, among which the Raninae and the Dicroglossinae, the latter including the tribe Paini. The taxon Paini was first proposed by Dubois (1992), as a tribe of the Raninae, for species that were further classified into two genera, *Chaparana* and *Paa*. These species are distributed from Afghanistan to southern China through the Himalayan region from northern Pakistan to northeastern India (through Nepal and Bhutan), and the mountains of the Indochinese region (Myanmar, Thailand, Cambodia, Laos, Vietnam). About ten years later, about 40 species are recognized in this tribe, including a few recently described species (Chen and Jiang, 2002, 2004; Chen *et al.*, 2002, 2004; Dubois, 2002). About half of them are found in China.

On the basis of molecular evidence, Dubois *et al.* (2001) transferred the tribe Paini from the subfamily Raninae to the Dicroglossinae. Dubois (2003) followed this classification. Independently, Jiang and Zhou (2005), on the basis of morphological and molecular evidences, suggested that the genus *Nanorana* (including the subgenus *Altirana*), placed by Dubois (1992) in the tribe Ranini, was a member of the tribe Paini, and that the latter clustered with the genera *Fejervarya*, *Hoplobatrachus* and *Limnonectes* of the subfamily Dicroglossinae, thus supporting the transfer of the tribe Paini to the subfamily Dicroglossinae. This was also supported by the results of Roelants *et al.* (2004). In the light of these results, the tribe is very probable monophyletic.

Duellman (1993) followed the taxonomic proposals of Dubois (1992) and grouped all species of the tribe Paini into

---

* Corresponding author. Phone: +33-1-4079-3485;
Fax : +33-1-4079-3488;
E-mail: adubois@mnhn.fr

[†] Present address: Museum of Vertebrate Zoology, University of California, Berkeley, Department of Integrative Biology, 3101 Valley Life Sciences Building #3160, Berkeley, CA 94720-3160, USA

Table 1. Species and specimens used in this study. The specimens are kept in the following collections: Chengdu Institute of Biology, Chinese Academy of Sciences, Sichuan, China (CIB); Kohima Science College, Kohima, Nagaland, India (KSC); Muséum National d'Histoire Naturelle, Paris, France (MNHN); Museum fur Tierkunde, Dresden, Germany (MTKD). The generic allocation of the species in the first column (and in the text) follows Dubois (1992, 2003), whereas the generic allocation in the second column is according to Fei (1999). For the valid names of the species here called *Fejervarya multistriata*, *Hoplobatrachus chinensis* and *Chaparana aenea*, see Dubois and Ohler (2000), Kosuch *et al.* (2001) and Dubois and Ohler (in press), respectively

Species and generic allocation according to Dubois (1992, 2003)	Generic allocation according to Fei (1999)	Voucher number	Locality of collection
Outgroup			
Hoplobatrachus chinensis (Osbeck, 1765)	*Hoplobatrachus*	CIB FY9806	Haikou (110°19'E, 20°03'N), Hainan, China
Limnonectes fujianensis Ye, Fei & Hu, 1993	*Limnonectes*	CIB 980028	Nanjing (117°21'E, 24°31'N), Fujian, China
Fejervarya multistriata (Hallowell, 1861)	*Fejervarya*	CIB F97056	Nanjing (118°47'E, 32°04'N), Jiangsu, China
Ingroup			
Chaparana aenea (Smith, 1922)	–	MNHN 1999.5818	Fan Si Pan (103°50'E, 22°15'N), Lao Cai, Vietnam
Chaparana quadranus (Liu, Hu & Yang, 1960)	*Paa*	CIB WX9801	Wuxi (109°36'E, 31°28'N), Sichuan, China
Chaparana unculuanus (Liu, Hu & Yang, 1960)	*Paa*	CIB 20025020	Jingdong (100°54'E, 24°28'N), Yunnan, China
Nanorana parkeri (Stejneger, 1927)	*Nanorana*	MNHN 2003.0004	Kampa (88°32'E, 28°18'N), Xizang, China
Nanorana pleskei Günther, 1896	*Nanorana*	CIB F97034	Hongyuan (102°42'E, 32°46'N), Sichuan, China
Paa boulengeri (Günther, 1889)	*Paa*	CIB F96030	Hongya (103°25'E, 29°56'N), Sichuan, China
Paa bourreti (Dubois, 1987)	–	MNHN 1999.5833	Sapa (103°50'E, 22°21'N), Lao Cai, Vietnam
Paa conaensis (Fei & Huang, 1981)	*Paa*	CIB 20020458	Cona (91°59'E, 27°59'N), Xizang, China
Paa exilispinosa (Liu & Hu, 1975)	*Paa*	MNHN 2003.0003	Hong Kong (114°11'E, 22°15'N), China
Paa liebigii (Günther, 1860)	*Paa*	CIB20020467	Yadong (88°54'E, 27°29'N), Xizang, China
Paa mokokchungensis (Das & Chanda, 2000)	–	KSC 341	Mokokchung (94°32'E, 26°20'N), Nagaland, India
Paa robertingeri (Wu & Zhao, 1995)	*Paa*	CIB F97007	Hejiang (105°46'E, 28°50'N), Sichuan, China
Paa rostandi (Dubois, 1974)	–	MTKD45060	Central area (83°32'E, 28°33'N), Nepal
Paa shini (Ahl, 1930)	*Paa*	CIB 2001L0027	Longsheng (110°00'E, 25°48'N), Guangxi, China
Paa spinosa (David, 1875)	*Paa*	CIB 980067	Tianmu Shan (119°36'E, 30°31'N), Zhejiang, China
Paa taihangnica Chen & Jiang, 2002	–	CIB 02070102003	Jiyuan (112°35'E, 35°08'N), Henan, China
Paa verrucospinosa (Bourret, 1937)	–	MNHN 2000.2942	Tam Dao (105°38'E, 21°28'N), Vinh Phuc, Vietnam
Paa yei Chen, Qu & Jiang, 2002	–	CIB SC-F	Shangcheng (115°24'E, 31°48'N), Henan, China
Paa yunnanensis (Anderson, 1879)	*Paa*	CIB 200043	Leibo (103°34'E, 28°15'N), Sichuan, China

the two genera, i.e., *Chaparana* and *Paa*, whereas Fei *et al.* (1991) and Fei (1999) grouped all the species of Paini from China into only one genus *Paa*. This classification within the tribe Paini was based on phenetic analyses of morphological data. To get further, it is necessary to get genetic information, such as DNA sequences of some genes, and to carry out phylogenetic analyses that will be used as basis for classification (Hillis *et al.*, 1996; de Queiroz, 1997; Avise and Johns, 1999). In the light of these data, the evolution of the morphological characters can be re-analyzed.

The present work is a first attempt to address the question of the phylogenetic relationships within the tribe Paini. For this work we used partial sequences of mitochondrial 12S and 16S rRNA genes of 19 species, including two species of the genus *Nanorana*, three of *Chaparana*, and 14 of *Paa* (see Table 1).

MATERIALS AND METHODS

Species used in this study

Tissues were obtained from nineteen species of the tribe Paini. Table 1 provides information on the voucher specimens and their collection localities.

Choice of the outgroup

In Jiang and Zhou (2005)'s results about phylogenetic relationships among several groups of Chinese Ranidae, the dicroglossine genus *Limnonectes* (represented by *L. fujianensis*) appeared to be the sister group of the tribe Paini (represented by three species of the genus *Paa* and two species of the genus *Nanorana*). Roelants *et al.* (2004) found that the tribe Paini is the sister group of a lineage containing the two genera *Fejervarya*, *Hoplobatrachus* and other related genera. Therefore, we incorporated *L. fujianensis*, *F. multistriata* and *H. chinensis* in the analysis as outgroup.

DNA amplification and sequencing protocols

Genomic DNA samples were obtained from ethanol-preserved tissues by extraction in a solution of hexadecyl-trimethyl-ammonium bromide (CTAB) buffer following the protocol provided by Winnepenninckx *et al.* (1993) with modifications.

Two regions of the mtDNA 12S and 16S rRNA genes were amplified and sequenced using the following protocols. Double stranded fragments were amplified in 35 cycles of PCR: 95°C for 30 s , 53–57°C for 30 s, 72°C for 60 s. It was pre-denatured at 95°C for 4 min before starting the cycles and elongated at 72°C for 7 min after ending the cycles. The PCRs were accomplished with the primer pairs of 12S (L1091: 5'-GCTTCAAACTGGGATTAGATAC-CCCACTAT-3', H1478: 5'-TGACTGCAGAGGGTGACGGGCGGT-GTGT-3') (Kocher *et al.*, 1989) that can amplify 388 base pairs, and 16S (L: 5'-CGCCTGTTTACCAAAAACAT-3', H: 5'-CCGGTCT-GAACTCAGATCACGT-3') (Simon *et al.*, 1994) that can amplify 576 base pairs. The capitals L and H indicate the amplified directions of light and heavy strand, respectively. After amplification, the PCR product was cleaned using Montage PCR Centrifugal Devices (Millipore). The cleaned DNA template was sequenced directly in both directions using CEQ2000 Sequencer (Beckman, Inc.).

DNA sequence analysis

Sequence alignment was conducted using Clustal W (ver. 1.6; Thompson *et al.*, 1994), and minor modifications were made by eye. The sequences from the two genes were combined as one data set for further analyses. Indel sites resulting from the alignment and missing/ambiguous sites were excluded from phylogenetic analyses.

Maximum parsimony (MP) and maximum likelihood (ML) as implemented in PAUP 4.0b8a (Swofford, 1998) and neighbor joining (NJ; Saitou and Nei, 1987) as implemented in MEGA (version 2.1; Kumar *et al.*, 2001) were employed to infer relationships among taxa. We inferred the best fitting model of sequence evolution using Modeltest 3.06 (Posada and Crandall, 1998) to be the general time-reversal model (GTR+I+G). Base frequencies were found unequal (A=0.3178, C=0.2422, G=0.1954, T=0.2446). The following parameters were set in ML analysis: Nst=6, Rmat=(4.0058 9.2365 3.8130 0.9225 23.5744), Rates=gamma, Shape=0.4878, Pinvar=0.2203. We used this model for the ML analysis.

The robustness of the phylogenetic results was tested by bootstrap proportion (BP; Felsenstein, 1985) with 1000 replicates in the NJ analysis and with 100 replicates in the MP and ML analyses.

RESULTS

Sequence characteristics and genetic distance between taxa

Sequences alignment resulted (Appendix 1) in a data matrix of 796 characters excluding indel sites, of which 311 sites were variable and 174 were parsimony informative. Nucleotide compositions were A 30.5, G 20.8, C 24.6 and T 24.0, and the average ratio of transitions to transversions was 1.8.

The levels of divergence expressed as the Kimura-2-parameter distances ranged from 0.140 (*P. chinensis* vs *P. yei*) to 0.255 (*F. multistriata* vs *P. liebigii*) between outgroup and ingroup, with an average of 0.191±0.014. Within the ingroup, they ranged from 0.018 (*P. spinosa* vs *P. exilispinosa*) to 0.130 (*P. boulengeri* vs *C. unculuanus*), with an average of 0.080±0.028.

Phylogenetic evaluation

The maximum parsimony analysis resulted in four shortest trees of 731 in length (CI=0.584, RI=0.571), the strict consensus tree is shown in Fig. 1. The result of the permutation tail probability (PTP; Faith and Cranston, 1991) test was P=0.01 (with 100 replicates), which indicates that these trees are significantly shorter than those from random data sets reproduced from the original data. The Paini taxa examined are divided into two quite well supported major groups: Group I (BP=99%) was composed of twelve species belonging to the three genera *Nanorana*, *Chaparana* and *Paa*, and Group II (BP=72%) comprising seven species of the genus *Paa*. Within Group I, three clades show high support: the clade formed by the two species of the genus *Nanorana*, the clade *C. aenea* with *C. unculuanus*, and the clade *P. yunnanensis* with *P. bourreti*. *P. rostandi* clustered with *P. liebigii* with moderate support (BP=60%). The cladogram shows *C. quadranus* as sister taxon to the clade *P. yunnanensis* and *P. bourreti* with moderate support (BP=61%). The position of the remaining species and nodes were left unresolved according the 50 % majority rule. Within the Group II, *P. yei* was located at the basal position; *P. verrucospinosa* is the sister taxon to the clade *P. boulengeri* and *P. robertingeri*; the third lineage recovered is the clade of *P. spinosa* and *P. exilispinosa*.

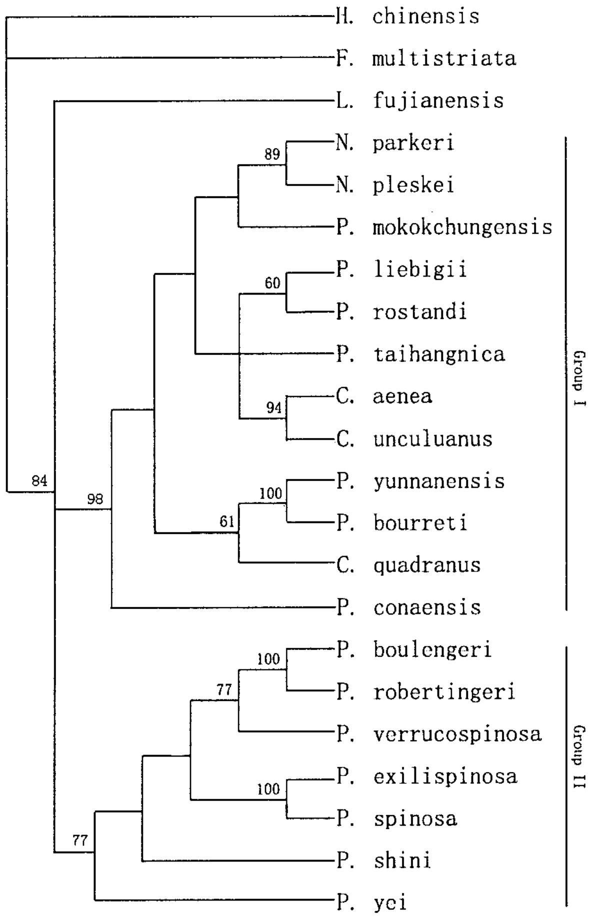

Fig. 1. Strict consensus of four shortest trees recovered in maximum parsimony analysis (tree length=731, CI=0.584, RI=0.571). Numbers on branches represent bootstrap support (100 replicates), omitted when the bootstrap value lower than 50%.

Relationships differed somewhat under the ML analysis

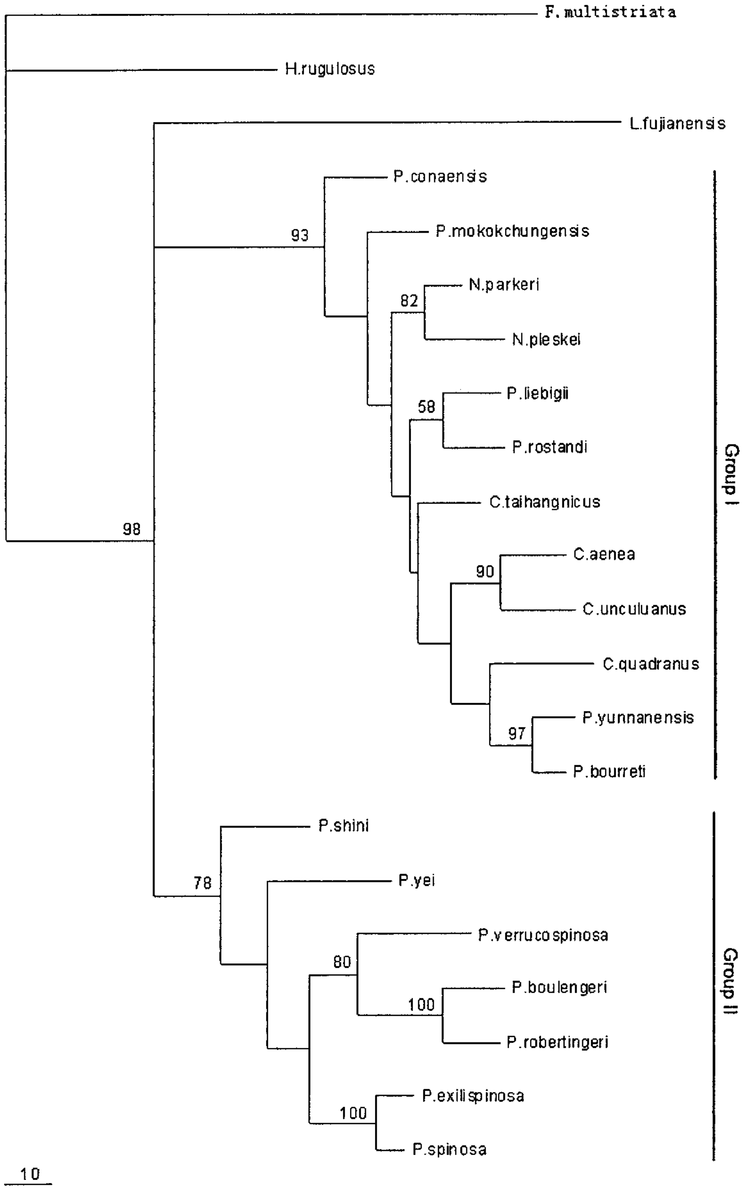

Fig. 2. The best tree derived by ML analysis Addseq=asis, lnL (unconstrained)=2854.672. Numbers on branches represent bootstrap support (100 replicates), omitted when the bootstrap value lower than 50%.

(Fig. 2) and the NJ analysis (Fig. 3). In both results, two identical major groups were recognized with high supports, the phylogenetic relationships among or between the species recognized in the MP analysis have higher support. Within the Group II, the phylogenetic relationships between the seven species studied in this work were well resolved except for *P. yei* and *P. shini*.

DISCUSSION

The MP, ML and NJ analyses suggest a monophyletic clade with two major groups for the 19 species examined. The genus *Nanorana* appears to belong to one of these two major clades. Our analysis indicates that these species of high-altitude frogs, which are also characterized by unique skeletal characteristics (Boulenger, 1920; Stejneger, 1927), and therefore were placed in two genera *Nanorana* and *Altirana*, are closely related to each other when considering morphological (Dubois, 1992) and molecular data (Jiang and Zhou, 2001). Our results confirm furthermore that the genus *Nanorana* is closely related to *Paa* as indicated by Boulenger (1920) and belongs in the tribe Paini as suggested by Roelants *et al.* (2004) and Jiang and Zhou (in press).

Details of the grouping, however, do not agree with those inferred from morphological evidence (Boulenger, 1920; Fei *et al.*, 1991; Dubois, 1992; Fei, 1999). Fei *et al.* (1991) and Fei (1999) included all species of the tribe Paini from China, except those of the genus *Nanorana*, in a single genus *Paa* and subdivided them into three subgenera, two

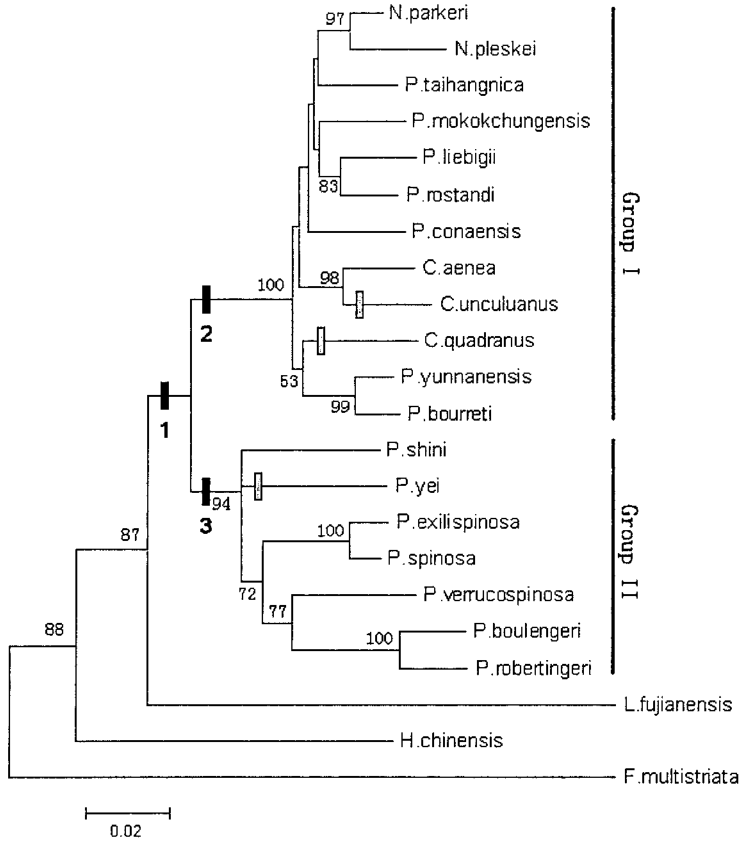

Fig. 3. The phylogenetic relationships among 19 species of the tribe Paini examined in the present study by NJ analysis. Numbers on branches are bootstrap proportions (1000 replications), omitted when the bootstrap value lower than 50%. Black bars indicate presence of large sized horny spines: 1 - presence of such spines; 2 - two separated patches of spines on breast; 3 - a single patch of spines covering breast and parts of belly. Grey bars indicate loss of horny spines on breast and belly of adult males.

of which are monotypic, i.e., *P.* (*Unculuana*) and *P.* (*Feirana*). The results of our work do not support this generic and infrageneric taxonomy. Dubois (1992) classified the members of the tribe Paini, except those of the genus *Nanorana*, into two genera *Chaparana* and *Paa*, both of which comprised four subgenera. Our mitochondrial data do not support this classification, in particular as the genus *Paa* does not appear to be monophyletic.

Within the subfamily Dicroglossinae, defined by skeletal characters (large nasal bones with almost no space between their inner edges, sphenethmoid invisible from dorsal view), the tribe Paini is characterized by the precoracoid unforked at the base, and keratinized spines on chest, belly, finger(s) and/or cloaca of adult male (exceptionally absent). These characters are shared by members of *Nanorana* supporting its relationship to *Paa* (Boulenger, 1920; Jiang, 1999).The two major subgroups recognized in this work appear justified in the light of their current distribution. Group I is mostly distributed in the western part, usually at high altitudes, of the range of the tribe Paini, whereas the group II is mostly distributed in the eastern part, at lower altitudes, although the distributions of the two groups overlap partially. The common ancestor of the tribe Paini existed possibly ~60Ma (Roelants *et al.*, 2004), that is before the Indian plate collided with Eurasia (~40Ma, Patriat and Achache, 1984). This collision caused the rapid rise of the Himalayan mountain range, creating many barriers for the dispersal of these frogs and facilitating rapid speciation in this group.

The presence of keratinized spines on the belly skin is a synapomorphy of the tribe Paini (Fig. 3). Two different distribution patterns of the spines can be distinguished: either spines form two patches on the chest, or spines are scattered as a unique patch more or less on the whole ventral region. These two patterns correspond to the two groups I and II recognized here on the basis of molecular data. Within the Group II the main pattern can be subdivided into four arrangements of the keratinized spines: absent on chest, but present on cloaca (*P. yei*), on chest and lateral sides of belly (*P. shini*), covering most of surface of belly (*P. boulengeri*, *P. robertingeri* and *P. verrucospinosa*) and present on chest and upper part of belly (*P. exilispinosa* and *P. spinosa*). These morphological differences are consistent with existence of the four lineages recognized from our molecular data set. But it is difficult to infer evolutionary history of the character transformation, since the phylogenetic relationship among to the last three groups remains unresolved (Fig. 1).

Or analysis include three species of the genus

Chaparana sensu Dubois (1992), mainly defined by the absence of large sized horny spines in adult males. Two of them, *C. aenea* and *C. unculuanus*, were assigned to the same subgenus *Chaparana* (*Chaparana*), and this classification is supported by our results. On the other hand, the results of the MP, ML and NJ analyses consistently showed that *C. quadranus* is not closely related to *these two species,* and should not be included in the subgenus *Chaparana*. Absence of horny spines in adult males is clearly a homeoplasic character which appeared at least three times in Paini (Fig. 3). Definition of *Chaparana* calls for revision.

This study provides partial support to two of the subgenera recognized by Dubois (1992) in his genus *Paa*: *Paa* (*Gynandropaa)* for *yunnanensis* and *bourreti*; *Paa* (*Paa)* for *liebigii* and *rostandi*. However, two other species, *P. conaensis* and *P. mokokchungensis*, assigned to the later subgenus by Dubois (1992, 2002), do not firmly cluster with *P. liebigii* and *P. rostandi*. On the other hand, this study provides strong support to the subgenus *Paa* (*Quasipaa*) recognized by Dubois (1992), for *P. boulengeri*, *P. exilispinosa*, *P. shini* and *P. spinosa*, as well as for the recently described or re-described species *P. robertingeri* (Wu and Zhao, 1995), *P. verrucospinosa* (Bourret, 1937; Inger *et al*., 1999) and *P. yei* (Chen *et al*., 2002, 2004). This lineage is also well characterized by morphological and biogeographical information briefly mentioned above.

The species *mokokchungensis* was described as a new species of the megophryid genus *Scutiger* by Das and Chanda (2000), but later transferred based to the ranid genus *Paa* by Dubois (2002) on morphological evidence. This allocation of the species is supported by the results of the present study. However, the relationships of this species with the other members of the tribe still have to be clarified.

In conclusion, our results suggest that the generic classification of the tribe Paini should be modified. In order to recognize taxonomically the consistent high support of the lineage and the morphological and molecular differentiation of Group II (Fig. 1–3), the subgenus *Paa* (*Quasipaa*) should be raised to the generic level. The status of the other groups, previously recognized as *Chaparana*, *Gynandropaa*, *Nanorana* and *Paa*, whose relationships within Group I are not clarified, is still uncertain. More species and additional molecular markers need to be studied for further evaluating the phylogenetic relationships and the taxonomic status of these groups.

ACKNOWLEDGMENTS

We thank L. Fei, Y. Z. Huang, C. Li, S. Q. Li and J. Meren Ao for their help in collecting samples that made this study possible. We are grateful to S. Tillier, E. Pasquet and N. Vidal for their help in the Molecular Systematics Laboratory of the Paris Muséum National dHistoire Naturelle. This work was supported by NSFC (No. 30000018) and Life Science Special Fund of Chinese Academy of Sciences (CAS) by the Ministry of Finance (STZ-01-19) to J. P. Jiang and by field work fund from the PPF “Faune et Flore de l’Asie du Sud-Est” of the Muséum National d’Histoire Naturelle.

REFERENCES

Avise JC, Johns GC (1999) Proposal for a standardized temporal scheme of biological classification for extant species. Proc Natl Acad Sci USA 96: 7358–7363

Boulenger GA (1920) A monograph of the south Asian, Papuan, Melanesian, and Australian frogs of the genus *Rana*. Rec Ind Mus 20: 1–126

Bourret R (1937) Notes herpétologiques sur l’Indochine française. XIV. Les batraciens de la collection du Laboratoire des Sciences Naturelles de l’Université. Descriptions de quinze espèces ou variétés nouvelles. Annexe Bull Inst publ Hanoi 1937: 5–56

Chen XH, Jiang JP (2002) A new species of the genus *Paa* from China. Herpetologica China 9: 231

Chen XH, Jiang JP (2004) Supplementary description of *Paa* (*Feirana*) *taihangnicus* (Ranidae, Anura) from Taihang mountain, Henan of China. Acta Zootax Sin 29: 595–599

Chen XH, Jiang JP, Qu WY (2004) Description of *Paa* (*Feirana*) *yei* (Anura, Ranidae) from China. Acta Zootax Sin 29: 381–385

Chen XH, Qu WY, Jiang JP (2002) A new species of the subgenus *Paa* (*Feirana*) from China. Herpet China 9: 230

Das I, Chanda S (2000) A new species of *Scutiger* (Anura: Megophryidae) from Nagaland, North-eastern India. Herpetol J 10: 69–72

De Queiroz K (1997) The Linnean hierarchy and the evolutionization of taxonomy, with emphasis on the problem of nomenclature. Aliso 15: 125–144

Dubois A (1992) Notes sur la classification des Ranidae (Amphibiens, Anoures). Bull mens Soc Linn Lyon 61: 305–352

Dubois A (2002) The taxonomic status of *Scutiger mokokchungensis* Das & Chanda, 2000 (Amphibia, Anura). Amph-Rept 22: 499–501

Dubois A (2003) True frogs (Ranidae). In: Hutchins M, Duellman WE and Schlager N (ed.), Grzimek’s animal life encyclopedia, 2nd ed, Vol 6, Amphibians, Farmington Hill, Michigan, Gale Group, pp 245–264

Dubois A, Ohler A (2000) Systematics of *Fejervaya limnocharis* (Gravenhorst, 1829) (Amphibia, Anura, Ranidae) and related species. 1. Nomenclatural status and type-speciemens of the nominal species *Rana limnocharis* Gravenhorst, 1829. Alytes 18: 15–50

Dubois A, Ohler A (2005) Taxonomic notes on the Asian frogs of the tribe Paini (Ranidae, Dicroglossinae). I. Morphology and synonymy of *Chaparana aenea* (Smith, 1922), with proposal of a new statistical method for testing homogeneity of small samples. J Nat Hist (in press)

Dubois A, Ohler A, Biju SD (2001) A new genus and species of Ranidae (Amphibia, Anura) from south-western India. Alytes 19: 53–79

Duellman WE (1993) Amphibian species of the world: additions and corrections. Univ Kans Mus Nat Hist Spec Publ 21: 1–372

Duellman WE, Trueb L (1985) Biology of amphibians. New York, McGraw-Hill, “1986”: i-xix + 1–670

Faith DP, Cranston PS (1991) Could a cladogram this short have arisen by chance alone? : on permutation tests for cladistic structure. Cladistics 7: 1–28

Fei L (1999) Atlas of Amphibians of China. Zhengzhou: He’nan Science and Technology Press

Fei L, Ye CY, Huang YZ (1991) Key to Chinese Amphibia. Chongqing: Chongqing Branch, Science and Technology Literature Publishing House

Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39: 783–791

Hillis DM, Moritz C, Mable BK Eds (1996) Molecular Systematics, Second Edition. Sinauer Assoc., Inc., Sunderland

Inger RF., Orlov N & Darevsky I (1999) Frogs of Vietnam: A report on new collections. Fieldiana. Zool NS 92: 1–46

Jiang JP (1999) Studies on systematics of the family Ranidae in China. Ph. D. dissertation, Nanjing Normal University, Jiangsu Province, China

Jiang JP, Zhou KY (2001) Evolutionary relationships among chinese ranid frogs inferred from mitochondrial DNA sequences of 12S rRNA gene. Acta Zool Sin 47: 38–44

Jiang JP, Zhou KY (2005) Phylogenetic relationships among chinese ranids: inferred from sequence data set of 12s and 16s rDNA. Herpet J 15: 1–8

Kocher TD, Thomas WK, Meyer A, Edwards SV, Pääbo S, Villablanca FX, Wilson AC (1989) Dynamics of mitochondrial DNA evolution in mammals: Amplification and sequencing with conserved primers. Proc Natl Acad Sci USA 86: 6169–6200

Kosuch J, Vences M, Dubois A, Ohler A, Böhme W (2001) Out of Asia: mitochondrial DNA evidence for an Oriental origin of tiger frogs, genus *Hoplobatrachus*. Mol Phyl Evol 21: 398–407

Kumar S, Tamura K, Jakobsen IB, Nei M (2001) MEGA2: Molecular Evolutionary Genetics Analysis software, Arizona State University, Tempe, Arizona, USA.

Patriat P, Achache J (1984) India-Eurasia collision choronology has implication for crustal shortening and driving mechanism of plate. Nature 311: 615–625

Posada D, Crandall KA (1998) MODELTEST: testing the model of DNA substitution. Bioinformatics 14: 817–818

Roelants K, Jiang JP, Bossuyt F (2004) Endemic ranid (Amphibia: Anura) genera in southern mountain ranges of the Indian subcontinent represent ancient frog lineages: evidence from molecular data. Mol Phyl Evol 31: 730–740

Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4: 406–425

Simon C, Frati F, Beckenbach A, Crespi B, Liu H, Flook P (1994) Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Ann Ent Soc Am 87: 651–701

Stejneger L (1927) A new genus and species of frog from Tibet J Washington Acad Sci 17: 317–319

Swofford DL (1998) PAUP*, Phylogenetic Analysis Using Parsimony (* and other methods). Sinauer, Sunderland, MA, Version 4.0

Thompson JD, Higgins DG, Gibson DJ (1994) CLUSTALw: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. Nucleic Acids Res 22: 4673–4680

Winnepenninckx B, Backeljau T, De Wachter R (1993) Extraction of high molecular weight DNA from molluscs. Trends Genet 9: 407

Wu GF, Zhao EM (1995) A new ranid species of the spinosae group from Sichuan. In: “Amphibian zoogeographic division of China” ed by EM Zhao, Sichuan J Zool 15: 52–55

(Received May 10, 2004 / Accepted January 13, 2005)

Appendix 1. The alignment result of sequences data used in this work. Specific names are those appearing in the first column of Table1: see text for the generic allocation of these species following the taxonomic changes tentatively proposed in the conclusion.

								80
F. multistriata	CTACGATTCT	CTGCCCGGAC	TACGAGCTTA	GTTAAATCCC	AAAGGACTTG	ACGGGTCCCA	CCCACTAGGA	GCCTGTCTAT
H. chinensis	.C..AC.A.C	.C...A....	.......C..	......A...	....	....	....	......T...
L. fujianensis	....ATCA.A	AC.G....TT	.......A..	......A...	....	....	....	......T...
N. parkeri	....ACCA.C	AC......T.	.......CC.	......A...	....	....	...C......	......T...
N. pleskei	....ACCA.C	AC......T.	.......CC.	......A...	....	....	...C......	......T...
P. liebigii	....ACCATC	AC......T.	.......CC.	......A...	....	....	...C......	......T...
P. rostandi	T...ACCATC	AC......T.	........C.	......A...	....	....	...C......	......T...
P. taihangnica	....ACCA.C	AC......T.	.......CC.	......A...	....	....	...C......	......T...
P. mokokchungensis	....ACCA.C	AC...A..T.	.......CC.	......A...	....	....	...C......	......T...
C. aenea	....ACCA.C	AC......T.	.......CC.	......A...	....	....	...T......	......T...
C. unculuanus	....ACCA.C	AC......T.	.......CC.	......A...	....	....	...T......	......T...
P. conaensis	....ACCA.C	AC......T.	.......CC.	......A...	....	....	T..C......	......T...
P. yunnanensis	....ACCATC	AC......T.	.......CC.	......A...	....	....	T..C......	......T...
P. bourreti	....ACCATC	AC......T.	.......CC.	......A...	....	....	T..C......	......T...
C. quadranus	....ACCATC	AC......TT	.......CC.	......A...	....	....	...C......	......T...
P. boulengeri	....ATCA.C	AC...A..TT	....	......A...	....	....	T..C......	......TC..
P. robertingeri	....ATCA.C	AC...A..TT	....	......A...	....	....	T..C......	......TC..
P. exilispinosa	A...ACCA.C	AC...A..TT	...A...C..	......A...	....	....	T..C......	......TC..
P. spinosa	T...ACCA.C	AC...A..TT	...A...C..	......A...	....	....	T..C......	......TC..
P. verrucospinosa	....ACCATC	AC...T..TT	....	......A...	..G.......	....	T..C......	......TC..
P. shini	T...ACCA.C	AC...A..TT	.......C..	...G..A...	....	....	T..C......	......TC..
P. yei	.CC.CCCC.C	AC...A..TT	....	......A...	....	....	...T......	......T.T.

```
                                                                                                    160
F. multistriata     AATCGATGGT CCCGTATACC CAACCTTTTT TGCCATAGTC TGTTACCTCC GTCGCAATCT ACCATATGAA TGATTAGTTG
H. chinensis        .......AAC .....TC... .....CCCC. GCTT....C. ........T. ....T..C.C G..G...... .ATC..AG..
L. fujianensis      ....TT.AAC .......C.. ..C..CCCCA AC.AC...C. .A........ ..A.A..CT. .......... .CCC.TCAA.
N. parkeri          ........A. ......A... .....CCCC. ...A....C. .......... ....T.GCT. .......... ..C.A...A.
N. pleskei          ........A. ......A... ......CCC. ...A....C. .......... ....T.GCT. .......... ..T.C...A.
P. liebigii         .......AA. ......A... .....CCCC. ...A...AC. .......... ....T.GCT. .......... ..CC....A.
P. rostandi         .......AA. ......A... .....CCCC. ...AG...C. .......... ....T.GCT. .......... ..CC....A.
P. taihangnica      .......AA. ......A... .....CCCC. ...A....C. .......... ....T.CCT. ....C....G ..CCC...A.
P. mokokchungensis  .......AAC ......A... .....CCCC. C..A....C. .......... ....T.GCT. .......... ..C.A...A.
C. aenea            ........A. ......A... ......CCC. ...A....C. .......... ......GCT. .......... ..C.....A.
C. unculuanus       .......AA. ......A... ......CCC. ...A....C. .......... ....T.GCT. .......... ..C.....A.
P. conaensis        .......AA. ......A... .....CCCC. ...A....C. .......... ....T.GCT. .....G.... ..TCC...A.
P. yunnanensis      .......AA. ......A... .......C.. ...A....C. .......... ....T.GCT. .......... ...C....A.
P. bourreti         .......AA. ......A... ......CC.. ...A...... .......... ....T.GCT. .......... ...CC...A.
C. quadranus        .......AA. ......A... ......CCC. ...A....C. .......... ....T.GCT. .........G ..CCC...A.
P. boulengeri       C......AA. ......C... T....ACCC. ...A...... .......... ....T..CT. .....G...G ..C.C...A.
P. robertingeri     C......AA. ......C... T....ACCC. ...A...... .......... ....T..CT. .....G.... ..C.C...A.
P. exilispinosa     .......AA. .......... T....ACCC. ...A...... .......... ....T..CT. .......... ..C.C...A.
P. spinosa          .......AA. .......... T....ACCC. ...A...... .......... ....T.GCT. .......... ..C.C...A.
P. verrucospinosa   C......AAC .......... T....ACCC. ...A....C. .......... ....T..CT. .....G.... ..C.....A.
P. shini            .......AA. .......... T....ACCC. ...A...... .......... ......GCT. .......... ..C.....A.
P. yei              .......AA. ......C... T....ACCC. ...A...... .......... ....T..CT. .......... ..TC....G.

                                                                                                    240
F. multistriata     TCAAGACTTC AGACGTAGGT CAGGGCAACA TGAAAAGGAA GGAGGGCTCA ATTTTAACTA AACAAACGAA ATACTGCTGA
H. chinensis        AT...GTCC. .AC....... .......G.. ..GGGT.... .A........ ..C....... .......... G.........
L. fujianensis      .A..AGTACA .AC....... .......GT. ...TGG.... .A........ .......A.. ....T..... ..G.......
N. parkeri          .T...GTAC. ..T....... .......GT. ..GGGC.... .A........ .......T.. .......... .C...T....
N. pleskei          .T...GTAC. .AT....... .......GT. ..GGGT.... .A........ .......T.. .......... .C...T....
P. liebigii         CT..AGTGCT .AT....... .......GT. ..GGGC.... .A........ .......T.. .......... .C...T....
P. rostandi         CT..TG.ACT .AT....... .......GT. ..GGGG.... .A........ .......... .......... .C...T....
P. taihangnica      GT.....A.. .AT....... .......GT. ..G.G..... .A........ .......T.. .......... .C...T....
P. mokokchungensis  .T...G.AC. .AT....... .......GT. ..GGGT.... .A........ .......T.. .......... .C...T....
C. aenea            C......AC. .AT....... .......GT. ...GG..... .A........ .......... .......A.. .C...T....
C. unculuanus       C......AC. .AT....... .......GT. ...GG..... .A........ .......... .......... .C...T....
P. conaensis        C..G.G.AC. .AT....... .......GT. ..GGGC.... .A........ .......... .......... .C........
P. yunnanensis      CA....TAC. .AT....... .......GT. ...G...... .A........ .......T.. .......... .C........
P. bourreti         CA....TCC. .AT....... .......GT. ...G...... .A........ .......T.. .......... .C........
C. quadranus        C....G.AC. .AT....... .......GT. ..G.G..... .A........ .......T.. .......A.. .C........
P. boulengeri       .T...G..C. .AT....... .......GT. ..GGGC...G .A........ ......TA.. .......T.. .CG.......
P. robertingeri     .T...G..C. .AT....... .......GT. ..GGGC...G .A........ ......TA.. .......... .CG.......
P. exilispinosa     .T...G.AC. .AT....... .......GT. ...G.T..GG AA........ G.....TA.. ..T....... .CG.......
P. spinosa          .T...G.GC. .AT....... .......GT. ...G.T..GG AA........ G.....TA.. .......... .CG.......
P. verrucospinosa   .T...GT.C. .AT....... .......GT. ..GGGC...G .A........ ......TA.. ....G..... .CG.......
P. shini            .T.....AC. .AT....... .......GT. .A.GGT.... .A........ ......TA.. .......... .C........
P. yei              .......GC. .AT....... .......GT. C.TGGT.... .A........ ......CA.. .......... .CG.......

                                                                                                    320
F. multistriata     GACACAGGAA AGGAGGTTTA GAAGTGAGAA AAATAACGAG TTTCTTTAAC ATGGCCTGGG ACTCTTGTCC TACCGCCATG
H. chinensis        A......TC. .......... .T...A.A.. ..G..G..T. ...T...... .C........ ......A... AG......GT
L. fujianensis      A......CC. ...C...... .T...A.A.C G.TC.TA... CC.T.....T .A........ ....C.A... AG......GT
N. parkeri          A..T...TT. ...C...... .T...A.A.. ..G..G..T. ...T...... TA........ ....C.A... AG......GT
N. pleskei          ...T...TT. ...C...... .T...A.A.. ..G..G..TA ...T...... TA........ ......A... AG......GT
P. liebigii         A..T...TT. ...C...... .T...A.A.. ..G..G..T. ...T...... TA........ ....C.A... AG......GT
P. rostandi         A..T...TT. ...C...... .T...A.A.. .....G..T. ...T...... TA........ ....C.A... AG......GT
P. taihangnica      A..T...TT. ...C...... .T...A.A.. ..G..G..T. ...T...... TA........ ....A.A.A. AG......GT
P. mokokchungensis  A..T...TT. ...C...... .T...A.A.. ..G..G..T. ...T...... TA........ ..AT..A... AG......GT
C. aenea            A..T...TT. ...C...... .T...A.A.. .....G..T. ...T...... TA........ ......A... AG......GT
C. unculuanus       A..T...TC. ...C...... .T...A.AG. ..G..G..T. ...T...... TA........ ......A... AG......GT
P. conaensis        A..T...TT. ...C...... .T...A.... ..G..G..T. ...T...... TG........ ....C.A... AG......GT
P. yunnanensis      A..T...CT. ...C...... .T...A.A.G .....G..T. ...T...... TA........ ......A... AG......GT
P. bourreti         A..T...CT. ...C...... .T...A.A.. .....G..T. ...T...... TA........ ....C.A... AG......GT
C. quadranus        A..T...CT. ...C...... .T...A.A.. ..G..G..T. ...T...... TA........ ..GA..A..T AG......GT
P. boulengeri       A.T....CC. ...C...... .T...A.A.. .....GT.T. ...T...... .......... ......AC.T AG....AG.C
P. robertingeri     A.T....CC. ...C...... .T...A.A.. .....G..T. ...T....C. TA........ .....CAC.T AG.......C
P. exilispinosa     A......CC. ...C...... .T...A.A.. .....GT.T. ...T...... TA........ ......A..T AG......GT
P. spinosa          A......CC. ...C...... .T...A.A.. .....GT.T. ...T...... TA........ ......A..T AG......GT
P. verrucospinosa   A.T....CC. ...C...... .C...A.A.. .....G..C. ...T.....T .A........ ......A... AG......GT
P. shini            A......TC. ...C...... .T...A.A.. .....G..T. ...T...... TA........ ......T..T AG......GT
P. yei              A......CC. ...C...... .T...A.A.. .....G..T. ...T...... TA........ ....C.A... AG......GT
```

```
                                                                                                     400
F. multistriata    ATACGATTAA CGCGTCATCT GCCGTACAGC AGCAGATAAT CACTTGTCTT TAGCAGTACG CATGCGCGGC ATCACGAGGT
H. chinensis       .C.T.T.A.. .....ACC.. .....G.GAA G.AGC..... ........C. ..AT..CTT. T...AA.... .........G
L. fujianensis     ...TAT.A.. .....ACC.. .....G..AA G.AGC..... .........C ..AT.ACT.. T..CAA.... .........G
N. parkeri         .C.T.T.A.. .....AT... .....G.GAA G.AGC..... .......... ..AT..CTA. T..CAA.... .........G
N. pleskei         .CTT.T.C.. .....AT... .....G.GAA G.AGC..C.. A......... ..AT..CTA. T..CGA.... .........G
P. liebigii        .CGT.T.A.. .....ATC.. .....G.GAA G.AGC..... ..T....... ..AT..CTA. T..CAA.... .........G
P. rostandi        .C.T.T.A.. .....ATC.. .....G.GAA G.AGC..... .......... ..AT.ACTA. T..CAA.... .........G
P. taihangnica     .CTT.T.A.. .....ATC.. .....G.GAA G.AGC..... .......... ..AT.ACTA. T..CAA.... .........G
P. mokokchungensis .C.T.T.A.. .....ATCT. .....G.GAA G.AGC..... .......... ..AT..CTA. T..CAA.... .........G
C. aenea           .C.T.T.A.. .....AT... .....G.GAA G.AGC..... .......... ..ATGACTA. T..CAA.... .........G
C. unculuanus      ...TAT.A.. .....AT... .....G.GAA G.AGC..... .......... ..AT.ACTA. T..CAA.... .........G
P. conaensis       .C.T.T.A.. .....ATC.. .....G.GAA G.AGC..... .......... ..ATCACTA. T..CAA.... .........G
P. yunnanensis     .C.G.T.A.. .....AT... .....G.GAA G.AGC..... .......... ..ATGACTA. T..CAA.... .........G
P. bourreti        .C.T.T.A.. .....AT... .....G.GAA G.AGC..... .......... ..AT.ACTA. T..CAA.... .........G
C. quadranus       .C.T.T.A.. .....AT... .....G.GAA G.AGC..... .......... ..AT.ACTA. T..CAA.... .........G
P. boulengeri      .CTT.T.GTT ...A.ACC.. .....G.GAG GTAGC..... T......... ..AT.ACTA. T..CAA.... .........G
P. robertingeri    .CTTAT.GTT TC.A.ACC.. .....G.GAA G......... T......... ..AT.ACTA. T..CTA.... .........G
P. exilispinosa    .C.T.T.A.. .....ACC.. .....G.GAA G.AGC..... .......... ..AT.ACTA. T..CAA.... .........G
P. spinosa         .C.T.T.A.. .....ACC.. .....G.GAA G.AGC..... .......... ..AT.ACTA. T..CAA.... .........G
P. verrucospinosa  .C.T.T.A.. .....ACC.. .....G.GAA G.AGC..... .......... ..AT.ACTA. T..CAA.... .........G
P. shini           .C..AT.A.. .....ATC.. .....G.GAA G.AGC..... .......... ..AT.ACTA. T..CAA.... .........G
P. yei             ...TAT.A.. .....ACC.. .....G.GAA G.AGC..... .......... ..AT.ACTA. T..CAA.... .........G

                                                                                                     480
F. multistriata    CTCACTCACA CCCTTCCCAA TCAGTGAACT GATCTCCCCG GAAGAAGCGG GGATAAAAAT ATAAGACGAG AAGACCCCAT
H. chinensis       ..TTACTG.T ..T..T.... .......... .......... .......... ....G.T... .......... ..........
L. fujianensis     T.ATACTG.T ...CAT.T.. .......... ..CT...... .....G.... .A..GCT... .C........ ..........
N. parkeri         T.ATACTG.T ..TC.T.... .......... .......... .......... .....GTC.. .......... ..........
N. pleskei         T.ATACTG.T .GTG.T.T.. .......... .......... .......... .....GTTC. .......... ..........
P. liebigii        T.ATACTG.T ..TC.T.... .......... .......... .......... .......TC. .......... ..........
P. rostandi        T.ATACTG.T ..TC.T.... .......... .......... .......... .......T.. .......... ..........
P. taihangnica     T.ATACTG.T ..TC.T.... .......... .......... .......... .......TC. .......... ..........
P. mokokchungensis T.ATACTG.T ..TC.T.... .......... ...T...... .......... .......CC. .......... ..........
C. aenea           T.ATACTG.T ..TC.T.... .......... ...T...... .......... ......GTC. .......... ..........
C. unculuanus      T.ATACTG.T ..TC.T.... .......... ...T...... .......... ......GCC. .......... ..........
P. conaensis       T.ATACTG.T ..TC.T.... .......... .......... .......... .......CC. .......... ..........
P. yunnanensis     T.ATACTG.T ..TC.T.... .......... .......... .......... .......CC. .......... ..........
P. bourreti        T.ATACTG.T ..TC.T.... .......... .......... .......... .......CC. .......... ..........
C. quadranus       T.ACACTG.T ..TCCT.... .......... .......... .......... .......CC. .......... ..........
P. boulengeri      T.ACACTG.T ..TCCT.... .......... .......... .......... .......... .......... ..........
P. robertingeri    T.ACACTG.T ..TCCT.... .......... .......... .......... .......... .......... ..........
P. exilispinosa    T.ATACTG.T ..TCCT.... .......... A......... .........A ......G... .......... ..........
P. spinosa         T.ATACTG.T ..TCCT.... .......... A..T...... .......... ......G... .......... ..........
P. verrucospinosa  T.ACACTG.T ..TCCT.... .......... A......... .......... .......G.. .......... ..........
P. shini           T.ACACTG.T ...CCT..G. .......... .......... .......... .....G.... .......... ..........
P. yei             T.ATACTG.T ..TCC..... .......... A......... .......... ......T... .......... ..........

                                                                                                     560
F. multistriata    GGAGCTTTAA ACCCAATAGA CACCTGATCT CCCAAAACAT TCTAAGTTTG TCTATTGGTT TTAGGTTGGG GTGACCACGG
H. chinensis       .......... ......CGAC AC..CC.C.C ....TTTT.. .GC.CACC.. .TCG...... .......... ......G...
L. fujianensis     .......... .......GTC A....AC.AA ..ACCT.A.A A.A..A.A.. ATC....... ..G....... ..........
N. parkeri         .......... ..T...C.AC A....CCCAA T..C.C.T.C CACG.ACC.. .T.G..A... ..C....... ......G...
N. pleskei         .......... ..T...C.AC A....CCCAA TT.TGT.T.C CACG.ACC.. .T.G..A... ..G....... ......G.A.
P. liebigii        .......... ..T...C.AC A.TT.CTAGA TT.T.C.T.. .A.G.AAC.. .T.G..A... ..G....... ......G...
P. rostandi        .......... ..T...C.AC A..T.CCCAA T..T.C.T.C C..G.AAC.. .T.G..A... ..G....... ......G...
P. taihangnica     .......... ..T...C.AC A..T.CCCAA TT.C.C.T.C .AAG.ACC.. CT.G..A... .......... ......G...
P. mokokchungensis .......... ..T...C.AC A....CCCAA TT.T.C.T.C ...G...C.. .T.G..A... ..G....... ......G...
C. aenea           .......... ..TT..C.AC A..T.C.CAA TT.TTC.T.. .A.G.A.C.. .T.G..A... .......... ......G...
C. unculuanus      .......... ..TT..C.AC A..T.CCCAA TT.T.C...C .A.G.ACC.. .T.G..A... ..G....... ......G...
P. conaensis       .......... ..T...C.AC A....CCCAA .T.C.C.T.. C.CG..CC.. ...G..A... .......... ......G...
P. yunnanensis     .......... ..T...C.AC A....TTCAA TA.C.C.T.. .A.G.ACC.. ...G..A... ..G....... ......G...
P. bourreti        .......... ..T...C.AC A..TCT.CAA TA.T...T.. .A.G.ACC.. ...G..A... ..G....... ......G...
C. quadranus       .......... ..T...C.AC A....T..AA T..T.CGT.C CGCG.A.C.. .T.G..A... ..G....... ......G...
P. boulengeri      .......... ......C.AC A....ATCAA ...CT..T.A .GCG.AAC.. ...G...... .......... ......G...
P. robertingeri    .......... ......C.AC A....ATCA. ...CTG.T.A .GCG..AC.. ...G...... .......... ......G...
P. exilispinosa    .......... ......C..C A....ATCAA T..CTG...C .AAG..CC.A C..G...... .......... ......G...
P. spinosa         .......... ......C..C A....ATCAA ...CT.GT.C .AAG..CC.. C..G...... .......... ......G.A.
P. verrucospinosa  .......... ......C.AC A....ATCAA .T.CT..T.C .AC...AA.. ...G...... .......... ......G...
P. shini           .......... ......C.AC A..TC.CAAC ...CTT.T.C .GC...AC.. CT.G...... .......... ......G...
P. yei             .......... ......C.AC A.T..AT.A. ...CTG.T.C .G.G..CC.. .T.G...... .......... ......G...
```

```
                                                                                                    640
F. multistriata    AGTACAATAA ATCCTCCATG ACGTACGGGA CTCCCTCTTA TCCTTGAACC ACATTTCTAA GAATCAACAA ATTGACGTTT
H. chinensis       ....T.GATT .A......C. ...A.T.... .A...C.... C..AA..G.T ..TCC..... .G.....A.. ........AA
L. fujianensis     ..A.A..C.. .A........ ...A.A..AC ..AATC..A. ..TAC..G.T ..TAC..C.. ......CT.. T.....A.A.
N. parkeri         ..A.A..CT. .A......C. ...A....A. TAA.TC..A. ..TAA..G.. ..CGC..... .......T.. ......A.AA
N. pleskei         ..A.A..CT. .A......C. ...A....A. TAA.TC..A. ..TAA..G.. ..CGC..... .......T.. ......A.AA
P. liebigii        ..A.A...T. .A......C. ...A.T..A. TAA.TC..A. ..TAA..G.. ..CGC..... .......T.. ......A.AA
P. rostandi        ..A.A...T. .A......C. ...A....A. .AA.TC..A. ..TAA..G.. ..CGC..... .......T.. ......A.AA
P. taihangnica     ..A.A..CT. .A......C. ...A....A. TAA.TC..A. ..TAA..G.. ..CGC..... .......T.. ......A.AA
P. mokokchungensis ..A.A...T. .A......C. .T.A.T..A. TAA.TC..A. .TAA..G..G .CGC...... ......T... .....A.AA.
C. aenea           ..C.A..CT. .A......C. .T.A.A..A. TAA.TC..A. ..TAA..G.. ..CGC..... .......T.G C.....A.AA
C. unculuanus      ..G.A..CTT .G......C. .T.A.T..A. .AA.TC..A. .T.AA..G.T ..CAC..... A......G.. ......A.AA
P. conaensis       ..A.A..CT. .A......C. .T.A....A. TAA.TC..A. ..TAA..G.. ..CAC..... .......T.. ......A.AA
P. yunnanensis     ..A.A..CT. .A......C. ...A....A. TAA.TC..A. ..TAA..G.. ..CAC..... .......T.. ......A.AA
P. bourreti        ..A.A..CT. .A......C. ...A....A. TAA.TC..A. ..TAA..G.A ..TAC..... .......T.. ......A.AA
C. quadranus       ..A.A..CC. .A......C. ...A....A. AAA.TC..A. ..TAA..G.T ..TAC..... .......... ......A.AA
P. boulengeri      ....A..CTT .G......C. .T.A...... TAA..C..A. ...AA..G.. ..CGC..... .......T.. ......A.GA
P. robertingeri    ....A..CTT .G......C. .T.A...... TAA..C..A. ...AA..G.. ..CGC..... .......... ........AA
P. exilispinosa    ....A..CT. .G......C. ...A...... TAA..C..A. ...AA..G.. ..CGC..... .......T.. ........AA
P. spinosa         ....A..CT. .A......C. ...A...... TAA..C..A. ...AA..G.. ..CGC..... .......T.. ........AA
P. verrucospinosa  ....A...TT .A......C. ...A...... TAA..C.... ...AA..GTT ..TAC..... .......... ........AA
P. shini           ....A..C.T .A......C. ...A.T.... TAA..C.... ...AA..G.. ..TGC..... .......T.. ........AA
P. yei             ....A..CTT .A......C. ...A...... TAA..C.... ...AA..G.. ..TGC..... .......T.. ........AA

                                                                                                    720
F. multistriata    TGATCCAATA ATTGATCAAC GGACCAAGTT ACCCTGGGGA TAACAGCGCA ATCCATTTAA GAGCCTATCG CCAAATGGGT
H. chinensis       ........GC T......... .......... .......... .......... .......... .......... A.........
L. fujianensis     ......GGC. .CC....... .A........ .......... .......... .......... .......... A.........
N. parkeri         ......G... ..C....... .......... .......... .......... .......... .......... A.........
N. pleskei         ......G... ..C....... .......... .......... .......... .......... .......... A.........
P. liebigii        ......G... ..C....... .......... .......... .......... .......... .......... A.........
P. rostandi        ......G... ..C....... .A........ .......... .......... .......... .......... A.........
P. taihangnica     ......G... ..C....... .......... .......... .......... .......... .......... A.........
P. mokokchungensis .....G.... .C........ A......... .......... .......... .......... .........A .........
C. aenea           ......G... G.C....... .......... .......... .......... .......... .......... A.........
C. unculuanus      ......G... ..C....... .......... .......... .......... .......... .......... A.........
P. conaensis       ......G... ..C....... .A........ .......... .......... .......... .......... A.........
P. yunnanensis     ......G... ..C....... .......... .......... .......... .......... .......... A.........
P. bourreti        ......G... ..C....... .A........ .......... .......... .......... .......... A.........
C. quadranus       ......G... ..C....... .A........ .......... .......... .......... .......... A.........
P. boulengeri      ......G.CT ..C....... .A........ .......... .......... .......... .......... A.........
P. robertingeri    ......G.C. ..C....... .A........ .......... .......... .......... .......... A.........
P. exilispinosa    ......G... ..C....... .A..T..... .......... .......... .......... .......... A.........
P. spinosa         ......G... ..C....... .A..T..... .......... .......... .......... .......... A.........
P. verrucospinosa  .......... .......... .A........ .......... .......... .......... .......... A.........
P. shini           ......G.C. ..C....... .A........ .......... .......... .......... .......... A.........
P. yei             ......G.C. ..C....... .A........ .......... .......... .......... .......... A.........

                                                                                               796
F. multistriata    TTACGACCTC AAGTTGGATC AGGGTATCCA GTGTGCAGCA GCTACTAATG GTTAGTTGTT CAACAATTAA AACCCT
H. chinensis       .......... .T........ ......C... .........C .......... ...T...... .......... ......
L. fujianensis     .......... .T........ .......... .......... ........A. ...T...... .......... ......
N. parkeri         .......... .T........ .......... .........C ........A. ...T...... .......... ......
N. pleskei         .......... .T........ .......... .........C ........A. ...C...... .......... ......
P. liebigii        .......... .T........ .......... .........C ........A. ...T...... .......... ......
P. rostandi        .......... .T........ .......... .........C ........A. ...T...... .......... ......
P. taihangnica     .......... .T........ .......... .........C ........A. ...T...... .......... ......
P. mokokchungensis .......... .T........ .......... .........C ........A. ...T...... .......... ......
C. aenea           .......... .T........ .......... .........C ........A. ...T...... .......... ......
C. unculuanus      .......... .T........ .......... .........C ........A. ...T...... .......... ......
P. conaensis       .......... .T........ .......... .........C ........A. ...T...... .......... ......
P. yunnanensis     .......... .T........ .......... .........C ........A. ...T...... .......... ......
P. bourreti        .......... .T........ .......... .........C ........A. ...T...... .......... ......
C. quadranus       .......... .T........ .......... .........C ........A. ...T...... .......... ......
P. boulengeri      .......... .T........ .......... .......... ........A. ...T...... .......... ......
P. robertingeri    .......... .T........ .......... .......... ........A. ...T...... .......... ......
P. exilispinosa    .......... .T........ .......... .........C ........A. ...T...... .......... ......
P. spinosa         .......... .T........ .......... .......... ........A. ...T...... .......... ......
P. verrucospinosa  .......... .T........ .......... .........C ........A. ...T...... .......... ......
P. shini           .......... .T........ .......... .........C ........A. ...T...... .......... ......
P. yei             .......... .T........ .......... .........C ........A. ...T...... .......... ......
```