A NEW SPECIES OF RANID FROG (AMPHIBIA: ANURA: RANIDAE) OF THE *HYLARANA SIGNATA* COMPLEX FROM PENINSULAR MALAYSIA

Kin Onn Chan1,5,6, Rafe M. Brown1, Kelvin K. P. Lim2, Norhayati Ahmad3, and L. Lee Grismer3,4

*1Biodiversity Institute and Department of Ecology and Evolutionary Biology, University of Kansas, Lawrence, KS 66045-7561, USA*

*2Raffles Museum of Biodiversity Research, Department of Biological Sciences,*

*National University of Singapore, 6 Science Drive 2, Singapore 117546, Republic of Singapore*

3*School of Environment and Natural Resource Sciences, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 Bangi, Selangor Darul Ehsan, Malaysia*

4*Department of Biology, La Sierra University, 4500 Riverwalk Parkway, Riverside, California, 92515-8247 USA*

[[1]](#footnote-2)Present Address: Department of Ecology and Evolutionary Biology, University of Kansas, Lawrence, KS 66045-7561, USA

Abstract:We describe a new species from the *Hylarana signata* Complex in Peninsular Malaysia based on morphological and genetic differentiation. The new species can be differentiated from its congeners by the following combination of characters: (1) adult males reaching 37.6 mm SVL; (2) nuptial pads absent (males); (3) humeral glands large (males); (4) webbing on toes reduced, not extending beyond middle subarticular tubercle of fourth toe; (5) dorsolateral stripe straight, unbroken, red or orange in color; (6) middorsal region black, unmarked; (7) flanks black, unstratified; (8) large, round, yellow spots on flanks, dorsal part of limbs and upper labia; (9) venter dark brown, whitish spots on throat, whitish reticulations on belly. The new species is phenotypically most similar to the distantly allopatric *H. siberu* (Siberut Island, south of Sumatra, Indonesia) but differs by having larger, more dense and more rounded spots on the flanks and dorsal side of limbs, and larger spots along the entire upper lip as opposed to smaller spots restricted to the proximal half of the upper lip. We use mitochondrial data (12S–16S ribosomal RNA gene fragments) to estimate genealogical relationships and genetic divergences between the new species, *H. siberu*, a related Sumatran population, and other members of the *H. signata* Complex. These data unequivocally support the recognition of the new taxon and provide insights into its evolutionary relationships.

*Key words:*  *debussyi*; *Hylarana*; *siberu*; Sumatra; systematics; taxonomy

The systematics of the *Hylarana signata* Complex has been historically perplexing due to similarities in their morphology (Boulenger, 1920; Inger, 1954; Zainudin and Sazali, 2012). This is most apparent in Peninsular Malaysia where the species names *H. signata* and *H. picturata* have been applied inconsistently and interchangeably over the years (Taylor, 1962; Berry, 1975; Brown and Guttman, 2002). Species from this complex are generally characterized as riparian forest frogs with a dark-brown to black dorsum covered with orange spots, and having a distinct orange dorsolateral stripe (Inger, 1954, 1966). Paradoxically, although interspecies variation may be somewhat conserved (Brown and Guttman, 2002), polymorphism within populations/species is pronounced enough to render the identification of diagnostic morphological characters for species delimitation problematic (Zainudin and Sazali, 2012).

The most recent study of this complex described high altitudinal populations of the *H. signata* Complex from the Malay Peninsula as a distinct species (*H. banjarana*) based on adult and larval morphological differences (Leong and Lim, 2003). Another distinct population was discovered from a lowland primary forest in central Peninsular Malaysia (Leong and Lim, 2004). Known from only one specimen at that time, it was designated as *H. siberu* (=*Rana siberu*), which at the time was only known from Siberut Island, off the southern coast of Sumatra (Dring et al., 1989). The hypothesized conspecificity of these two populations was based on them sharing the following characters: (1) entirely black dorsum without spots/blotches; (2) uninterrupted dorsolateral stripes from snout tip to vent, stripes red/deep orange in life; (3) lips, limbs and lower flanks with spots (vs. barrings), yellow in life; (4) males with enlarged humeral glands, paired subgular vocal sacs, without nuptial pads (Dring et al., 1989; Leong and Lim, 2004). Unfortunately, no tissue samples were taken from that specimen. An additional specimen was recently collected from the adjacent area (Chan and Norhayati, 2009), which allowed a more robust examination of the taxonomic and phylogenetic placement of these disjunct populations. Being fully cognizant of the pitfalls of describing new lineages based on just two specimens, we evaluate these populations within the framework of a lineage-based, Unified Species Concept (De Queiroz, 2005) and use morphological diagnosibility, phylogenetic relationships, genetic divergence, and geographic isolation as criteria for assessing lineage independence. Results from our analyses provide sufficient evidence to justify the recognition of this lineage as a new species, which is described herein.

Materials and Methods

*Sampling.—*Samples used for the molecular analyses include three for the outgroup and 19 for the ingroup. Outgroups were selected based on a prior study of the *signata* Complex (Brown and Guttman, 2002). The ingroup consists of four species that are endemic to the Philippines (*H. mangyanum, H. moellendorffi, grandocula,* and *H. similis*); *H. picturata* and *H. signata* that are widespread throughout the Malay Peninsula, Sumatra, and Borneo; *H. siberu* and an undescribed population from Sumatra; *H.* *banjarana* from the Malay Peninsula; and the new species from central Peninsular Malaysia (Table 2).

*Morphological characters.—*The following measurements were taken with Mitutoyo digimatic caliper to the nearest 0.1 mm: snout-vent-length (SVL), tip of snout to vent; head length (HL), posterior margin of lower jaw to tip of snout; head width (HW), taken immediately posterior to eyes; snout length (SL), anterior corner of the orbit to tip of snout; eye-nostril-distance (END), anterior corner of eye to posterior margin of nostril; nostril-snout-distance (NSD), anterior margin of nostril to tip of snout; internarial distance (IND), distance between nostrils; eye-to-eye-distance (EED), distance between anterior corners of both eyes; eye diameter (ED), distance between anterior and posterior corners of upper and lower eyelids; interorbital distance (IOD), distance across top of head between medial margins of orbits at their closest points; tympanum diameter (TD), horizontal width of tympanum at its widest point; tympanum-eye-distance (TED), shortest distance between anterior margin of tympanum and posterior corner of orbit; brachium length (BL), axilla to flexed elbow; forearm length (FAL), flexed elbow to base of inner metacarpal tubercle; femur length (FL), vent to outer margin of flexed knee; tibia length (TBL), outer margin of flexed knee to outer margin of flexed tarsus; tarsal length (TL), outer margin of flexed tarsus to base of inner metatarsal tubercle. Webbing formula follows Savage and Heyer (1997). The new species was morphologically compared to all species from the *H. signata* Complex and its relevant outgroups. Voucher specimens of comparative material are listed in Appendix 1 of Brown and Guttman (2002). The holotype has been deposited at the Raffles Museum of Biodiversity Research, Singapore; the paratype at University Kebangsaan Malaysia Herpetological Collection. Voucher abbreviations used are as follow: DWNP, Department of Wildlife and National Parks, Malaysia; FMNH, Field Museum of Natural History; KU, University of Kansas; ZRC, Zoological Reference Collection, Raffles Museum of Biodiversity Research, Singapore.

*Molecular data.—*Genomic DNA was extracted from liver using a modified Guanidine Thiocyanate extraction protocol developed by M. Fujita (Esselstyn et al., 2008). A 2434 nucleotide base-pair fragment of mitochondrial DNA that encodes part of the 12S–16S rRNA (and part of one flanking transfer RNA genes (tRNAval) gene was amplified in four fragments using the polymerase chain reaction (PCR) and thermal profiles and primers in Evans et al. (2003). We cleaned PCR products with ExoSAP-IT (USB) and sequenced cycle sequencing products in both directions on a 3130*xl* DNA Analyzer (Applied Biosystems) using the same primers and Big Dye v3 chemistry (Perkin Elmer). We assembled consensus sequences in Sequencher v. 4.1 (Genecodes), manually edited resulting contigs in McClade 4.07 (Maddison and Maddison, 2005) and excluded hypervariable regions for which unequivocal homology assessments could not be confidently ascertained. Data are deposited in GenBank under accession numbers XXXX–XX (Table 2).

*Phylogenetic analyses*

An unpartitioned maximum likelihood (ML) analysis was conducted in RAxML version 7.2.8 (Stamatakis, 2006) using the GTR + Γ + I model of nucleotide substitution as selected under the Akaike Information Criterion (AIC; implemented in jModeltest v0.1.1; Posada and Crandall, 2008), with 100 replicate best-tree inferences, employing a random starting tree for each inference. We assessed clade support with 1000 bootstrap replicates. GAMMA + P-Invar model parameters were estimated up to an accuracy of 0.001 Log Likelihood units. A Bayesian analysis was performed in MrBayes v3.2.1 (Ronquist and Huelsenbeck, 2003) under the same nucleotide substitution model. We employed two separate MCMC analyses, each with four Metropolis-coupled chains (temperature setting = 0.02; exponential distribution with a rate parameter of 25 as a branch length prior). We ran analyses for 10 million generations, with parameters and topology sampling every 2000 generations, discarding the first 25% as burn-in. Using the program Tracer v1.4 (Rambaut and Drummond, 2007), ESS values were inspected to evaluate stationarity and convergence. Pairwise distances were calculated using PAUP\* 4.0b10 (Swofford, 2002).

Results

*Phylogenetic Analyses*

Sequences included 554 (out of 1,015) variable characters for 12S + tRNAval and 753 (out of 1,435) for 16S. Resulting topologies recovered high bootstrap support (ML) and posterior probabilities, which were congruent across all nodes. Malaysian *Hylarana banjarana* was reconstructed as the first-diverging species in the *H. signata* Group species (Brown and Guttman, 2002). The next diverging lineage consists of the Siberut Island endemic *H. siberu*,the undescribed species from Peninsular Malaysia, and a related and undescribed high-elevation Sumatran species. The remaining *H. signata* Complex taxa fall into a clade from islands of Sundaland and the Philippines, with *H. picturata*, and *H. signata* estimated to be distantly related to the Peninsular Malaysian population (Fig. 1). Within its clade, the Peninsular Malaysian population is substantially genetically divergent from its closest relatives *H. siberu* (8% uncorrected *p*-distance) and *H.* sp. Sumatra (10%).

*Systematics*

Results from our phylogenetic analyses clearly demonstrate that the *Hylarana* from central Peninsular Malaysia is not conspecific with *H. siberu* but represents a genetically divergent lineage. We further provide morphological evidence to show that this population warrants specific recognition and is herein described as a new species.

Hylarana**centropeninsularis** *sp. nov.*

Figs. 2, 3A

*Rana siberu* Leong and Lim, 2004:261

*Hylarana siberu* Chan and Norhayati, 2009:295; Chan et al*.*, 2010:203

*Holotype*.—Adult male (ZRC1.10536; Fig. 2), collected by C.H. Lim on 17 March 2003 (ca. 2200 hrs) at Sungai Temir, within the Lakum forest reserve, Raub, Pahang, Malaysia (3°40’N, 101°55’E; 105 m above sea level; datum = WGS84).

*Paratype*.— Adult male (DWNP 1189; Fig. 3A), collected by Juliana Senawi on 27 April 2006 in pit-fall trap at Kuala Gandah, Pahang, Malaysia (3°35'N, 102°8'E; 90 m above sea level; datum = WGS84).

*Diagnosis*.—The new species can be differentiated from its congeners by the following combination of characters: adult males reaching 37.6 mm SVL; nuptial pads absent (males); humeral glands large (males); webbing on toes reduced, reaching middle subarticular tubercle of fourth toe but not beyond; dorsolateral stripe straight, unbroken, red in color; middorsal region black, unmarked; flanks black, unstratified; large, round, yellow spots on flanks, dorsal part of limbs and upper labia; venter dark brown, whitish spots on throat, whitish reticulations on belly.

*Comparison to other species*.—*Hylarana centropeninsularis* can be readily distinguished from *H. grandocula, H. mangyanum, H. moellendorffi, H. picturata, H. signata* and *H. similis* by the absence (vs. presence) of nuptial pads in males and a homogenous, unmarked middorsal region (vs. spotted/blotched). It can be further differentiated from *H. moellendorffi* and *H. picturata* by having a continuous (vs. broken) dorsolateral stripe. *Hylarana* *centropeninsularis* shares the aforementioned characters with *H. siberu* and *H.* sp. Sumatra, with which it is phylogenetically and morphologically most similar to, but differs by having larger, denser and more rounded spots on the flanks and dorsal side of limbs, and larger spots along the upper labia.

*Description of holotype.***—**Adult male, SVL 37.4 mm; head longer than wide (HL/HW=1.2), snout rounded, sloping anteroventrally, projecting beyond lower jaw, snout length equal to eye diameter; canthus rostralis distinct, loreal region concave, vertical; nostrils oval, located laterally, closer to canthus than supralabial, closer to rostrum than eye (NSD/END=0.7); internarial distance half of distance between front of eyes (IND/EED=0.6); eyes relatively large (ED/SL=1.0; ED/HL=0.4), eye diameter larger than interorbital distance (ED/IOD=1.9); tympanum and tympanic annulus distinct, oval, diameter smaller than eye (TD/ED=0.8), larger than distance to eye (TD/TED=3.4); supratympanic fold prominent, extending obliquely from posterior margin of tympanum to dorsal portion of front limb insertion area; choanae tear-drop shaped, diameter 0.8 mm, tapering medially, separated by distance larger than their diameter; vomerine teeth small and indistinct, numbering four, arranged in short oblique row atop the dentigerous process of vomer; vocal sacs paired, internal subgular; tongue elongate, widening posteriorly with a deep central terminal notch, free for one third its length.

Arms relatively long and slender (BL/SVL=0.2), brachial and forearm length subequal (BL/FAL=0.9); humeral glands on lateroventral part of brachium; order of fingers from shortest to longest: II–IV–I–III (fingers I and IV subequal); fingers without web; finger tips dilated into small, pointed discs bearing circummarginal grooves; subarticular tubercles prominently raised, oval, opaque; number of subarticular tubercles on each finger is given in parentheses following finger number denoted by Roman Numerals: I(1), II(1), III(2), IV(2); supernumerary tubercles indistinct, translucent, at the base of first phalanx on each finger; inner metacarpal tubercle large, oval, translucent; palmar tubercle oval, translucent, slightly smaller and not in contact with inner metacarpal tubercle; outer metacarpal tubercle elongate, translucent, in contact, same length, but half the width of palmar tubercle; nuptial pads absent (Fig. 2A).

Hindlimbs long, slender (FL/SVL=0.5; TBL/SVL=0.5; TL/SVL=0.3), tibia slightly longer than femur (TBL/FL=1.1); order or toes from shortest to longest: I–II–III–V-IV (toes III and V subequal); toe tips slightly dilated into small, pointed discs bearing circummarginal grooves; web formula: I ½ − 1 II 0+ −1½ III 0+ − 2- IV 2- − 0 V; subarticular tubercles prominently raised, translucent; number of subarticular tubercles on each toe is given in parentheses following toe number denoted by Roman Numerals: I(1), II(1), III(2), IV(3), V(2); inner metatarsal tubercle elongate, raised, translucent; outer metatarsal tubercle round, raised, translucent, smaller than inner (Fig. 2B).

*Color in life.***—**Dorsum completely black without markings; near complete orange dorsolateral stripe from rostrum, along the canthus, lateral margin of palpebrae, dorsolateral part of dorsum, and terminating at the sacrum where it forms a near complete loop; a single row of white spots along the upper and lower labials; flanks and dorsal side of limbs with round, creamy yellow spots; some spots connect to form short, elongated bars; venter dark brown; throat with whitish spots; belly with whitish reticulations.

*Color in preservative.***—**Dorsum dark brown; flanks and dorsal side of limbs a lighter shade of brown; dorsolateral stripe and spots/bars white; venter the same color as in life (Fig. 2C–D).

*Distribution.***—**The new species is currently known from two adjacent localities in central Peninsular Malaysia: Sungai Temir (=Temir River), Lakum Forest Reserve, Raub Pahang and Kuala Gandah, Lanchang, Pahang (Fig. 1).

*Natural History.***—**The holotype was caught in a pit-fall trap at least 100 m from the nearest stream in a lowland secondary forest (Chan and Norhayati, 2009), whereas the paratype was collected at night in an adjacent lowland primary forest from the edge of a temporary forest pool (ca. 1.5 x 1.0 m). This pool was among a swampy, waterlogged area away from streams. It was syntopic with the following anurans: *Hylarana labialis, H. erythraea, Polypedates macrotis, Rhacophorus appendiculatus, Philautus* sp. (Leong and Lim, 2004). These data suggest *H. centropeninsularis* is a swamp habitat specialist which stands in contrast to *H. picturata* (sensu Brown and Guttman, 2002) which is restricted to forest streams.

*Etymology.***—**The specific epithet is derived from the latin prefix “centro” (root=centrum) and the root “peninsularis,” in reference to the type and only known localities of the new species in central Peninsular Malaysia.

Discussion

We hypothesize *H. centropeninsularis* which is the sister species of *H. siberu* and *H.* sp. Sumatra, to be a species now exhibiting a relictual distribution. This assertion is based on phylogenetic affinity (Fig. 1), morphological similarity (Table 1) and geographic proximity (Fig. 1). One plausible scenario would be the existence of a widespread ancestral species that occurred in Sumatra and Peninsular Malaysia in the past. This is substantiated by the fact that the Strait of Malacca that separates Peninsular Malaysia and Sumatra is narrowest (ca. 65 km) and shallowest (ca. 40 m) at the central portion of Peninsular Malaysia and was land positive at or below 40 m below present-day levels for approximately 55% of the time in the last 17,000 years (Geyh et al*.*, 1979; Voris, 2000). The inundation of the Strait of Malacca could serve as the vicariant event that isolated the ancestral population(s), which subsequently diverged in isolation.

The new species has more in common with *H. siberu* in that both species occupy a generally similar ecological niche (lowland swamps) as opposed to *H.* sp. Sumatra, a montane species, which in that regard, is more similar to *H. banjarana* from Peninsular Malaysia (Leong and Lim, 2003). Given the small, patchy, microhabitat-specific, and apparently relictual nature of *H. centropeninsularis*, we would recommend prioritizing survey efforts in any identifiable lowland swamp habitats in the immediate near future. Not only are swamps and peat bog habitats insular in nature (surrounded by a generalized terrestrial habitat matrix), these habitats are heavily imperiled and rapidly disappearing throughout Southeast Asia (Ng et al., 1994; Myers et al., 2000). The possibility that additional new species could await discovery in these unique habitats around the world should not be ignored (Biton et al., 2013).

*Hylarana* sp. Sumatra was collected from the type locality of *H. debussyi* (van Kampen) at 1000 m elevation in the Batak Mountains, Bandar Baru, Sumatra (M. Kamsi and D. Iskandar, *personal communication*; Fig. 1), initially leading us to consider the possibility of the application of this name to the distinct *H. signata* Complex species from high elevations of northern Sumatra. However, further inspections into the descriptions of *H. debussyi* (=*Rana debussyi*) in Boulenger (1920), van Kampen (1923) and the plate from the original description of the species (van Kampen, 1910, p. 23, pl. I, fig. 3) revealed that *H. debussyi* did not share the characters that would warrant its inclusion in the *Hylrana signata* Complex (sensu Brown and Guttman, 2002). The following characters were given by van Kampen (1923): brown above; sides, from tip of snout to vent, black, with a white streak from the tip of the snout along the upper lip, below the tympanum to the thighs; limbs pale brown, with dark *cross*-bars; yellowish white beneath. Moreover, Boulenger (1920) described it as allied to *H. luctuosa*, but having much in common with *H. nicobariensis*. These discrepancies, along with results from our molecular analyses suggests that *H*. sp. Sumatra represents a distinct and undescribed lineage.

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Table 1*.***—**Morphometric measurements of the holotype and paratype in millimeters. See Materials and Methods for abbreviations.

|  |  |  |
| --- | --- | --- |
|  | ZRC 1.10536 | DWNP 1189 |
|  | (Holotype) | (Paratype) |
| Sex | Male | Male |
| SVL | 37.4 | 37.6 |
| HL | 14.0 | 15.2 |
| HW | 12.0 | 12.5 |
| SL | 5.9 | 6.4 |
| END | 3.7 | 4.2 |
| NSD | 2.5 | 1.8 |
| IND | 3.7 | 3.6 |
| EED | 6.7 | 7.2 |
| ED | 6.0 | 5.1 |
| IOD | 3.2 | 3.5 |
| TD | 4.8 | 2.2 |
| TED | 1.4 | 1.2 |
| BL | 7.3 | 8.0 |
| FAL | 8.1 | 8.2 |
| FL | 17.6 | 17.7 |
| TBL | 19.9 | 19.2 |
| TL | 12.9 | 10.6 |

Table 2*.***—**Samples used in this study and GenBank accession numbers. See Materials and Methods for museum abbreviations.

|  |  |  |  |
| --- | --- | --- | --- |
| **Species** | **Voucher** | **Locality** | **Genbank #** |
| *Hylarana glandulosa* | FMNH 248254 | Brunei, Belait District |  |
| *Hylarana baramica* | FMNH 249870 | Malaysia, Sabah, Beaufort District |  |
| *Hylarana baramica* | FMNH 248218 | Brunei, Belait District |  |
| *Hylarana* spSumatra | MK 334 | Northern Sumatra Island, Langkat, Bandar Baru, Batak Mountains |  |
| *Hylarana* spSumatra | MK 335 | Northern Sumatra Island, Langkat, Bandar Baru, Batak Mountains |  |
| *Hylarana centropeninsularis* | DWNP 1189 | Malaysia, Pahang, Kuala Gandah |  |
| *Hylarana siberu* | BJE 203 | Siberut Island, West Sumatra Province |  |
| *Hylarana siberu* | BJE 236 | Siberut Island, West Sumatra Province |  |
| *Hylarana picturata* | FMNH 235707 | Malaysia, Sabah, Kota Marudu |  |
| *Hylarana picturata* | ZRC 1.10886 | Borneo Island, Sabah, Mt. Kinabalu (Neotype Locality) |  |
| *Hylarana picturata* | FMNH 266930 | Sumatra Island, West Sumatra Province, Limau Manis |  |
| *Hylarana picturata* | FMNH 266944 | Sumatra Island, West Sumatra Province,Payakumbuh |  |
| *Hylarana signata* | FMNH 238842 | Mendolong, Sipitang Dustrict, Sabah, Malaysia |  |
| *Hylarana signata* | ZRC 1.12388 | Borneo Island, Sarawak, Matang |  |
| *Hylarana mangyanum* | KU 303566 | Philippines, Mindoro Island, Municipality of Paypayama, Barangay Carmundo, |  |
| *Hylarana mangyanum* | KU 303578 | Philippines, Mindoro Island, Municipality of Bongabong, Barangay Formon, |  |
| *Hylarana moellendorffi* | KU 309009 | Philippines, Palawan Island, Palawan Province, Municipality of Puerto Princesa City, Barangay Irawan |  |
| *Hylarana moellendorffi* | KU 327050 | Philippines, Palawan Island, Palwan Province, Municipality of Nara, Barangay Estrella Falls |  |
| *Hylarana grandocula* | KU 306492 | Philippines, Samar Island, Samar Proviunce Province, Municipality of San Jose de Baun, Barangay Poblacion |  |
| *Hylarana grandocula* | PNM 8848 | Philippines, Mindanao Island, Davao City Province, Municipality of Calinan, Barangay Malagos |  |
| *Hylarana similis* | TNHC 63007 | Philippines, Luzon Island, Camarines Norte Province, Municipality of Naga City, Barangay Panicuason, |  |
| *Hylarana similis* | PNM 5536 | Philippines, Luzon Island, Laguna Province, Municipality of Los Baños, University, of the Philippines campus, Mt. Makiling |  |

Fig. 1.—Maximum likelihood (ML) tree from RAxML analysis of 12S–16S rRNA (and part of one flanking transfer RNA genes (tRNAval) gene. Numbers above branches represent bootstrap support for ML/Bayesian posterior probabilities. Distribution ranges of species groups are color coded to correspond to the distribution map.

Fig. 2.—The holotype, showing A: Palmar view of manus; B: Plantar view of pes; C: Dorsum; D: Ventrum. Note greatly enlarged humeral gland (A, C) and reduced webbing of pes (B).

Fig. 3.—A:Paratype of *Hylarana centropeninsularis*; B: *Hylarana siberu*from Siberut Island, Sumatra (photo: J. A. McGuire); C: *Hylarana* sp Sumatra from northern Sumatra (photo: D. Iskandar); D: *Hylarana* (=*Rana*) *debussyi* takenfrom van Kampen (1910, p. 23, pl. I, Fig. 3).

1. 6 Correspondence: e-mail, kin\_onn@yahoo.com [↑](#footnote-ref-2)