Research Article

Three Species of Genus *Lepisiota* (Hymenoptera: Formicidae) with New Distributional Records from Khyber Pakhtunkhwa, Pakistan

Kiran Shahjeer[1]*, Gauhar Rehman[1], Khurshaid Khan[1] and Toheed Iqbal[2]

[1]Department of Zoology, Abdul Wali Khan University, Mardan, Khyber Pakhtunkhwa, Pakistan; [2]Department of Entomology, University of Agriculture, Peshawar, Khyber Pakhtunkhwa, Pakistan.

Abstract | A study on the ant fauna of north-western part comprising of the hilly colder mountains in the North to the Dry hot plains in the South of Khyber Pakhtunkhwa province of Pakistan was conducted during 2017-2020. Area was surveyed and collection of worker ants from the area was hired by using pitfall traps, aspirator and hand picking with the help of forceps. The study revealed that three species belonging to genus *Lepisiota* as a major outcome of this taxonomic study. The species include *L. frauenfeldi, L. simplex, L. opaca* and its sub-species *L. o. pulchella*. Among them two species viz., *L. simplex, L. opaca* and a subspecies of *L. opaca* i.e. *L. o. pulchella* are reported as first distributional records from Khyber Pakhtunkhwa. Keys to the subfamilies of Family Formicidae and worker caste species of genus *Lepisiota* collected from Khyber Pakhtunkhwa along with its spatial distributional map for all species and a sub-species is provided. All the specimen are deposited at Insect Museum, Department of Zoology, Abdul Wali Khan University Mardan, Pakistan. From the study, it is concluded that the fauna of Khyber Pakhtunkhwa is rich in ant species. Species diversity and abundance are expected more diverse collections in these and other similar areas. These findings can be strengthened by modern techniques of molecular identification.

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***Correspondence** | Kiran Shahjeer, Department of Zoology, Abdul Wali Khan University, Mardan, Khyber Pakhtunkhwa, Pakistan; **Email:** Kiranshahjeer@awkum.edu.pk
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Introduction

Ants are widely distributed, universally known, dominant group of individuals, grouped in family Formicidae, superfamily Vespoidea and order Hymenoptera of class Insecta. Globally ants acquired major position as keystone species, comprising 30% of faunal biomass in terrestrial environment. They evolved as more diverse and ecologically rich group among social insects (Majer, 1985). They are separated from other hymenopterans by presence of single or double petioles between alitrunk and gaster. Furthermore, they are classified in 15,000 species placed in 296 genera and 16 subfamilies (Bolton, 1994). Among them 12,000 are specified (Bolton *et al.*, 2006).

In geographical regions, Indo-Austalian region has large number of genera reported so far while African and Neotropical regions are more diverse and richer in endemic ant fauna than oriental and Nearctic region (Bolton, 1994). According to Bharti *et al.* (2016), about 828 species under 100 genera are reported only from India.

The tropical and continental forests have much more

diversity of ant fauna. Roughly these areas were documented to have about 2200 species reported only from Asia (Schmid-Hempel, 1992). The diversity of ants lowers gradually with ecological factors like latitudes, altitudes and aridity (Brener and Ruggiero, 1994).

Pakistan, a very important ecological zone of the oriental region has the biodiversity estimate far from conclusive. On other hand, there are serious issues of soil erosion, water logging, deforestation, and threats to other habitats, which badly effect biodiversity of region (Baig and Al-Subaiee, 2009). Khyber Pakhtunkhwa, a province of Pakistan, known earlier for its geographical position as North-West Frontier Province, situated at 34° 95 26 N and 72° 33 11 E, covering 101,741km^2. It is known well for its rich biodiversity, variety of habitats, varied environmental conditions and geologic history. It is situated in north west region of country along the border of Afghanistan. Geographically, the region is divided into two zones. Its northern zone is snowy and extremely cold in winters while summers are quite pleasant with heavy rainfall. However, its southern zone is hot and arid in summers and cold and dry in winters with very less rainfall. Dera Ismail khan, part of southern zone is even considered as one of the hottest regions of South Asia. Its climate varies a lot for a region of this much size, having most of the climate types found in Pakistan. Its snowy peaks, lush green valleys and dry hot plains present an unusual beauty with enormous biodiversity of life forms and climate

Ants are one of the important keystone species, occupying key position in our ecosystem. Its role cannot be denied because of its active involvement in our daily life. They form important group of social insects; their social behavior is mirror image of human social behavior. They can occupy almost every region of our world, presenting massive variation in geographic and ecological distribution. Its conservation needs its comprehensive information about distribution and taxonomy. That's why current study was planned to explore the distribution and taxonomical identification of variety of ant specimens collected from geographically and ecologically rich, biodiverse region of Khyber Pakhtunkhwa Pakistan, a home of very impressive diversity of life forms.

Materials and Methods

Area of study

Current study was performed on worker caste of ants belonging to Family Formicidae collected from different areas of KP, Pakistan. Ants were collected throughout the year during their active season. A map is provided showing main divisions of KP which were focused for survey i.e., Charsadda, Nowshera, Dera Ismail Khan, Bannu, Kohat, Peshawar, Mardan, Hazara, and Malakand. Collection from hilly areas of Galyat, Chitral, Waziristan, Bajaur and Dir was also included for collection of ants.

Methods of collection and identification

Standard insect collection was done with the help of aspirators and hand-picking during day and night time from dense vegetation, bushes, shrubs, weeds, small and tall grasses, bases and tops of tree trunks, agricultural fields, inside houses, roadsides, river sides, crevices, and from carcasses. Collected specimen were killed with the help of cotton swab soaked in ethyl acetate. Dead specimens were then kept in 70% ethanol for long time preservation. Preserved specimens were cleaned with the help of benzene, to remove dust and other body waxes. The specimens were kept in 90% ethanol 24 hours before mounting them on point cards or pinning them in thorax. All the specimen were identified mainly with the help of (Bingham, 1903, The fauna of British India, including Ceylon and Burma. Hymenoptera, Vol. II. Ants and Cuckoo-wasps. Bolton (1995), Bharti (2011, 2012), Bharti and Akbar (2013), Bharti *et al.* (2013), Wachkoo and Bharti (2014), Bharti *et al.* (2016), Akbar *et al.* (2017), Bodlah *et al.* (2017), Rasheed *et al.* (2019, 2020, 2021) were followed for identification of the collected ant specimen.

Equipment and software used

Nikon SMZ 745T stereo zoom trinocular microscope mounted with camera Nikon DS F-i2 was used for the identification and taking images of specimens. Stacking of important characters was done using Helicon Focus version 6.2.2. Nikon DS F-i2 was used for accurate measurements of the specimen.

Keys preparation

Keys to the subfamilies and species collected form Khyber Pakhtunkhwa is prepared from the most easily observable characters.

Tables and specimen preservation

A table showing spatial distribution of each species is also provided. The entire identified specimens are kept in Insect Museum, Department of Zoology, Abdul

Wali Khan University, Mardan. Additional specimens are deposited at Insect Museum, Department of Entomology, The University of Agriculture, Peshawar, Pakistan.

Abbreviations used

Following abbreviations are used in text;

Abbreviation used	Complete name	Abbreviation used	Complete name
Bi	Baragali	Kt	Kohat
Bjr	Bajaur	Lt	Lakki Marwat
Br	Buner	Ma	Mansehra
Bu	Bannu	Mkd	Malakand
Ch	Charsadda	Mn	Mardan
Chl	Chitral	Ni	Nathiagali
DIK	Dera Ismail Khan	Nrn	Naran
Dir	Dir	Nsr	Nowshehra
Gt	Abdominal/ Gaster segments	Pwr	Peshawar
Kgn	Kaghan	Sb	Swabi
Khr	Khanaspur	Sha	Shangla
KP	Khyber Pakhtunkhwa	Wa	Wana
Kk	Karak		

Results and Discussion

In Indo-Pak, the only significant work was done by Bingham (1903). His work was followed and it is still followed for taxonomic work of Indo-Pak region. No comprehensive work has been done in this part of the world after Bingham. In recent era Bharti (2011, 2012), Bharti and Akbar (2013), Wachkoo and Bharti (2014), Bharti *et al.* (2016) and Akbar *et al.* (2017) has initiated work on Indian ants. In Pakistan, traces of work have been started by Umair *et al.* (2012), Usman *et al.* (2017), Rasheed *et al.* (2019, 2020, 2021), Lakho *et al.* (2019) but still lot of research is needed to perform comprehensive work on ants of Pakistan.

Studying importance of ants and availability of scarce information regarding its taxonomy, we were compelled to explore biodiversity of Khyber Pakhtunkhwa, Pakistan. This baseline study will be helpful in future ant's conservation programs.

In current study, we have reported only 4 subfamilies (Formicinae, Ponerinae, Amblyoponinae and Myrmicinae) with 10 genera. Among these genera, only Genus *Lepisiota* bearing 3 species and a subspecies new to the distributional records of KP and Pakistan is discussed.

Key to the subfamilies of worker ants of Khyber Pakhtunkhwa is presented as follows.

Key to the subfamilies of family formicidae based on external morphology of workers caste, from Khyber Pakhtunkhwa, Pakistan

1. Eyes present ……..……..……..……..……..…….. 2
1′. Eyes absent……..……..……..…… Amblyoponinae
2. Body with a single node ……..……..……..…… 3
2′. Body with two nodes (petiole and post petiole) ……..……..……..……..……..……..…….. Myrmicinae
3. Abdomen with more or less marked constriction between basal two segments of abdomen; terminal end of abdomen with a rounded opening posteriorly (acidopore), acidopore with a sting and without a fringe of hairs surrounding it ……..……..……..……..……..……..…… Ponerinae
3′ Abdomen without any constriction between basal two segments of abdomen; terminal end of abdomen with a rounded opening posteriorly, acidopore nozzle shaped, without a sting and with or sometimes without a fringe of hairs surrounding it ……..……..……..…….. Formicinae

Subfamily formicinae

In current study, among 10 genera under the subfamily Formicinae have been encountered from various geographical locations of the province. Key to the species of genus *Lepisiota* under subfamily Formicinae of KP is given as follows.

Genus Lepisiota Santschi (1926)

- *Lepisiota* Santschi (1926a) PDF: 15 [as subgenus of *Acantholepis*]. Type-species*: Plagiolepis rothneyi*, by original designation.
- [*Lepisiota* first available replacement name for *Acantholepis* Mayr, 1861 (junior homonym of *Acantholepis* Kroyer, 1846: 98 (Pisces)), hence valid name of genus: Bolton, 1995b: 33.].
- *Lepisiota* in Formicinae, Plagiolepidini: Bolton, 1994: 51; Bolton, 2003 PDF: 23.
- [*Lepisiota* incorrectly as junior synonym of *Acanthomyrmex:* Brown (1973) and Snelling (1981). In both publications *Acanthomyrmex* is an error for *Acantholepis*.].
- Subgenera of *Lepisiota*: nominal plus *Lepisiota (Baroniurbania):* (Dietrich, 2004).

In current study, this genus comprises of 3 species encountered from different areas of KP. Key to the

species of this genus is given as below.

Key to the ant species belonging to the worker caste of genus Lepisiota based on morphological identification from Khyber Pakhtunkhwa, Pakistan

1. Antennal scape remarkably long, extending posterior margin of head more than half the length of antennal scape……. *frauenfeldi*
1′. Antennal scape not so long, extending posterior margin of head by not longer than one third its length ……..... 2
2. Node of pedicel with its upper lateral angles supported with short thick spines or teeth, pointing outwards, or node with emerginate upper part; abdomen apically pilose, head, thorax and abdomen smooth without any pilosity ……..... *simplex*
2′. Node of pedicel with its upper angles prevailed with fine, long, acute vertical spines; color and pilosity of head thorax and abdomen different ……..... 3
3. Abdomen purple, brown or black, without yellow markings ……..... *opaca*
3′. Abdomen dull black, the gaster segment 1 above and in front brownish yellow……..... *O. pulchella*

Lepisiota frauenfeldi Mayr (1855) (Plates 1-3)

- *Hypoclinea frauenfeldi* Mayr (1855) PDF: 378 (w.) Yugoslavia. Palearctic.
- Roger, 1859 PDF: 243 (q.); Emery, 1878: 46 (m.); Wheeler and Wheeler, 1968 PDF: 209 (l.).
- Combination in *Acantholepis*: Mayr, 1861 PDF: 42; in Lepisiota: (Urbani *et al.*, 1992).
- Senior synonym of *Lepisiota frauenfeldi azerbeidzhanica, Lepisiota caucasica*: (Arakelian, 1994).
- Bingham, 1903 PDF: 316; Santschi, 1917, PDF: 42; Tohmé, 1981 PDF: 1; Atanassov and Dlussky, 1992: 205.

Diagnostic characters: *L. frauenfeldi* can be separated from other species of the genus by the following characters; antennal scape extremely long, almost 1.5x longer than the posterior margin of apex of the head.

Material examined: (n= 23☿). Pakistan, KP., 3☿, Karak, 35 76 18N, 31 16 4E, 09.ix.2018, Najibullah. 3☿, Wana, 32 30 28N, 69 56 88E, 14.ix.2018, Najibullah. 3☿, Bannu, 32 98 61N, 70 60 41E, 09.ix.2018, Najibullah. 1☿, Bara Gali, 34 09 99N, 73 34 99E, 24.vii.2017, Fahad and Najibullah. 2☿, Nathiagali, 34 06 66N, 73 38 33E, 26.viii.2018, Najibullah. 2☿, Nizampur, 32.2433° N, 74.2619° E, 19.iii.2020. Toheed Iqbal. 1☿, Mansehra, 34 33 33N, 73 19 99E, 21.vii.2018, Fahad and Usman. 1☿, Kalam (Gabral), 35 52 50N, 72 41 25E, 03.vii.2018, Fahad and Usman. 3☿, Lakki Marwat, 32 25 08N, 70 47 18E, 1-3.v.2019, Toheed Iqbal. 2☿, Sarai Naurang (Gandhi Chowk), 32 70 13N, 70 78 03E, 1-2.v.2019, Toheed Iqbal. 1☿, Mardan, 34 12 00N, 72 03 10E, 27.iv.2019, Kiran and Marwa. 1☿, Dir Upper (Kumrat), 35 56 10N, 72 20 15E, 10.vii.2017, Fahad and Usman.

Distribution: The species is native to Palearctic region. It has also been reported from Algeria, Morocco, Azerbaijan, Iran, UAE, Bulgaria, Croatia, Greece, Italy, Malta, Spain, and Guam (Bolton, 2020).

Comments: This species was first reported by Mayr (1855) as *Hypoclinae frauenfeldi*. The species can be identified among other members of the genus *Lepisiota* by a long scape, almost 1.5x long, reaching behind the posterior margin of the head. It ranges in size from 2-3mm. Bingham (1903) reported it to be a variable species and marks some major differences with the species *Acantholepis bipartite.* Bolton (2020) reports 15 subspecies of this species. As in Pakistan, taxonomic work on Formicidae has not been so encouraging that we can rely on it, we therefore followed Bingham (1903) and Bolton (2020) for our identification. His work is still acceptable as it is such masterpiece that even after more than a century; his document is a source of identification in Indian subcontinent. The literature of Bolton (1994, 2003) is also helpful in removing confusion of synonyms. Moreover, the website of Bolton (2020) is also helpful in comparing the species photographs with the specimen. The website also provides access to the literature. From Pakistan this species has been reported before its creation by Bingham (1903). Umair *et al.* (2012) reported it from Rawalpindi district of Pakistan with the name *Acantholepis frauenfeldi* under subfamily Camponotinae, which shows that he strictly followed Bingham (1903). From KP this species is reported from district Charsadda and Swabi by Rasheed *et al.* (2020, 2021), respectively. Current research provides new distributional records for KP and Pakistan with detailed description, images and localities. As mentioned in Table 1, this species has been reported from Wana, Karak, Lakki Marwat, Mardan, Peshawar, Nowshera,

Table 1: *Spatial distribution of genus Lepisiota species and sub-species collected from different areas of Khyber Pakhtunkhwa, Pakistan during 2017-2020.*

Species	Pwr	Mn	Ch	Nsr	Sb	Chl	Ma	Kgn	Nrn	Bi	Ni	Swt	Khr	Dir	Sha	Bjr	Kt	Bu	Kk	Lt	Br	Wa	DIK	Mkd
L. frauenfeldi		+		+			+			+	+			+				+	+	+		+		
L. simplex					+							+							+	+				
L. opaca							+			+				+										
L. o. pulchella			+											+				+						

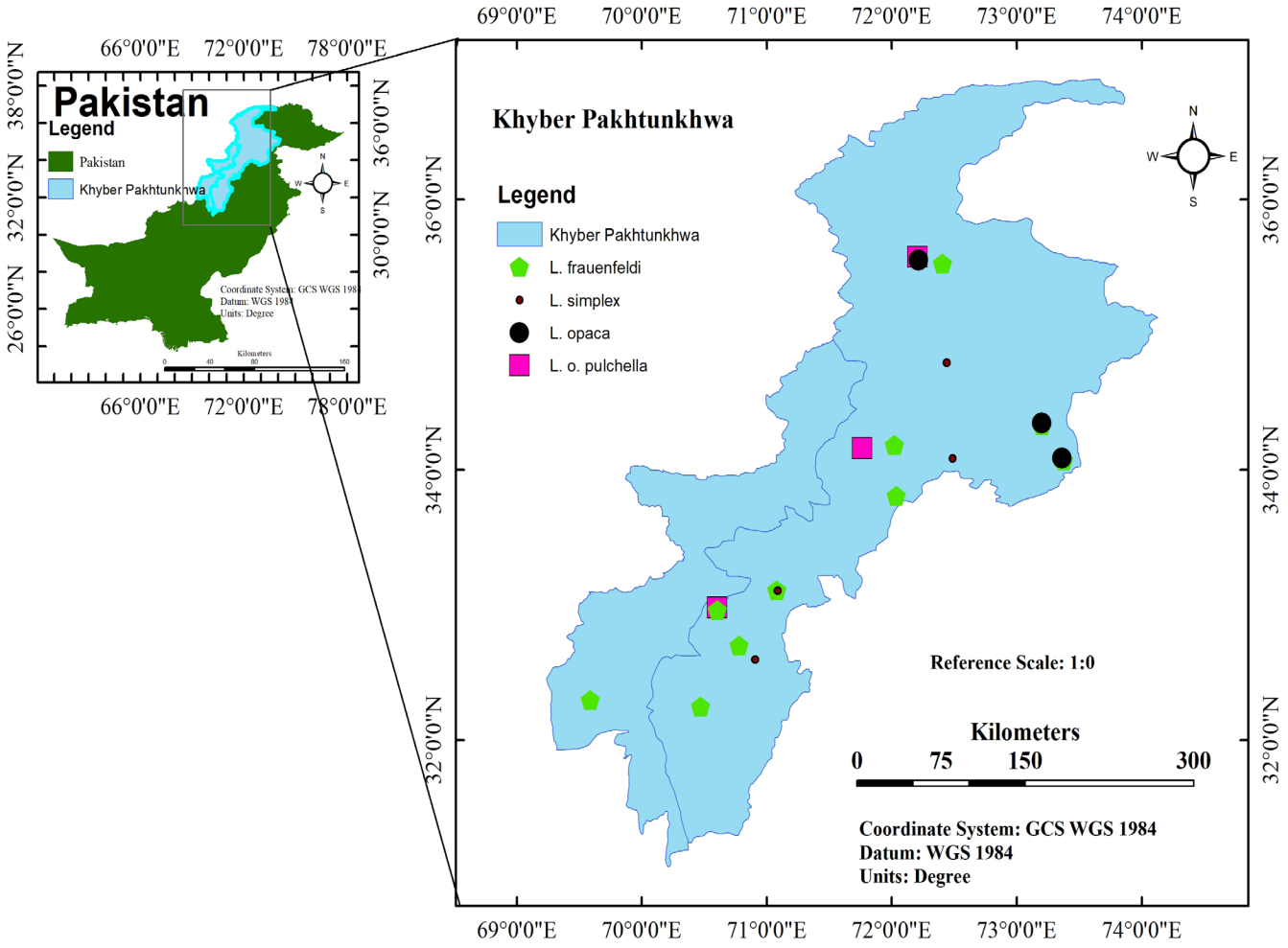

Figure 1: *Map of Khyber Pakhtunkhwa, Pakistan, showing spatial distribution of different worker species of genus Lepisiota.*

Baragali and Nathiagali in current studies which show that the species is spread over a wide area of KP. Spatial distribution of the species is given in map provided in Figure 1.

Lepisiota simplex Forel (1892) Plates (4-6)

- *Acantholepis simplex* Forel (1892a) PDF: 43 (diagnosis in key) (w.) Somalia. Afrotropic. Primary type information: Somalia, coll. Keller; CASENT0909878; MHNG
- Combination in *Lepisiota*: Bolton, 1995b: 228. Forel, 1892e PDF: 350 (q.m.).
- Status as species: Bingham, 1903 PDF: 317; Forel, 1910 PDF: 449; Forel, 1916 PDF: 438; Santschi, 1914 PDF: 378; Sharaf *et al.*, 2016 10.1080/00222933.2016.1180722 PDF: 1885.
- as subspecies of *Lepisiota capensis*: Forel, 1907i PDF: 86 (footnote); Arnold, 1920a PDF: 572; Wheeler, 1922: 937.

Diagnostic characters: The species differs from other members of the genus by characters mentioned as follows; overall body smooth without pilosity, except abdomen apically.

Material examined: (n= 7☿). Pakistan: KP.; 1☿, Karak, 35 76 18N, 31 16 4E, 09.ix.2018, Najibullah. 4☿, Kalam (Gabral), 35 52 50N, 72 41 25E, 03.vii.2018,

Fahad and Usman. 2☿, Swabi, 34 11 66N, 72 46 66E, 10.x.2018, Najibullah.

Distribution: Lesotho, Somalia (Africa), Saudi Arabia (Asia) (Bolton, 2020).

Comments: The species can be identified by entirely smooth body, apical margin of the abdomen pilose. The species was first reported by Forel (1892) from Afro tropical region. It is assumed to be sub species of *L. capensis* by Forel (1907), Arnold (1920), Wheeler (1922) after synonymizing it from *Acantholepis* to *Lepisiota* by Bolton (1995). The species is distributed in Africa and recently Sharaf *et al*. (2016) reported this species with a new status from Saudi Arabia. This species is collected with aspirator from Swabi, Swat, and Karak areas of KP. It shows that the species is distributed in plain, humid, warm and hilly areas of KP. Current species adds new distributional records to the knowledge of Formicidae of KP and Pakistan.

Lepisiota opaca Forel (1892) (Plates 7-9)

- *Acantholepis opaca* Forel (1892a) PDF: 43 (diagnosis in key) (w.) India. Indomalaya.
- Combination in *Lepisiota:* Xu, 1994: 235. Bingham, 1903 PDF: 318.

Diagnostic characters: *Lepisiota opaca* can be differentiated from other members of the genus *Lepisiota* by having purplish brown or black abdomen with yellowish markings (Bingham, 1903).

Material examined: (n= 15☿). Pakistan, KP., 1☿, Bara Gali, 34 09 99N, 73 34 99E, 24.vii.2017, Fahad and Najeeb. 9☿, Dir Upper (Kumrat), 35 56 10N, 72 20 15E, 10.vii.2017, Fahad and Usman. 5☿, Mansehra, 34 33 33N, 73 19 99E, 21.vii.2018, Fahad and Usman.

Distribution: China, India, Indomalaya (Bolton, 2020).

Comments: Morphologically the species differentiated from other members of the genus by having purplish brown or black abdomen with yellowish markings. According to Bingham (1903) species has been separated from very closely related *L. pulchella* by above mentioned character as well as the thorax is broader than *L. pulchella*, and region of mesonotum not so strongly constricted. This species was first reported by Forel (1892) under genus *Acantholepis*. Later the genus was replaced by *Lepisiota*, and hence the valid name till date remains *L. opaca*. This species seems to be native of Indian subcontinent as it is not reported elsewhere as mentioned above. Currently this species is encountered with the help of aspirator and hand picking with the help of forceps from hilly region of Hazara division (Mansehra and Baragali) and Gabral area of Swat division which meets Kumrat region of Upper Dir across the mountains. Bingham (1903), Bolton (1995) were followed for identification of genus and species. Additionally, species were confirmed by comparing with images provided by Bolton (2020).

Lepisiota opaca pulchella (Forel 1892) Plates (10-12)

- *Acantholepis opaca r. pulchella* Forel 1892a PDF: 43 (diagnosis in key) (w.) India (Maharashtra).
- Combination in *Lepisiota*: Bolton, 1995b: 228.
- Subspecies of *Lepisiota opaca*: Dalla Torre, 1893: 172; Forel, 1894: 414; Emery, 1925 PDF: 27; Chapman and Capco, 1951: 210; Bolton, 1995b: 228.
- Status as species: Bingham, 1903 PDF: 318; Wu, and Wang, 1995: 129; Zhou, 2001: 168.

Diagnostic characters: *L. o. pulchella* is sub-species of *L. opaca* but can be differentiated from it through the following characters; abdomen dull black, antero-dorsally the abdomen (Gt_1) brownish yellow; pronotum narrow; mesonotum with shallow or weak constriction anteriorly with pronotum.

Material examined: (n= 21☿). Pakistan, KP., 17☿, Dir Upper (Kumrat), 35 56 10N, 72 20 15E, 10.vii.2017, Fahad and Usman. 1☿, Bannu, 32 98 61N, 70 60 41E, 09.ix.2018, Najibullah. 3☿, Charsadda, 34 14 40N, 74 73 17E, 01.v.2018, Najibullah.

Distribution: India (Bolton, 2020).

Comments: Bingham (1903) in his key separated this species from similar in appearance with minute characters. In description of this species, he mentions its resemblance with *L. opaca*, but then explains the differences as well. Bolton (1995) synonymized this species under *Lepisiota*, which was *Acantholepis* earlier. This species is presented as sub-species of *L. opaca*. The species is reported only from India. Current study confirms its existence from hilly as well as plain areas of Khyber Pakhtunkhwa province of Pakistan. This sub-species is reported for the first time not only from KP but also from Pakistan.

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Novelty Statement

The baseline study was initiated in Khyber Pakhtunkhwa Pakistan to record and enlist, species of genus *Lepisiota*. In this research, three species were reported and one subspecies as new distributional records from different areas of Northwestern part of Pakistan.

Author's Contribution

Kiran Shahjeer: Principal author who conducted the research and wrote the article.
Gauhar Rehman and Khurshaid Khan: Supervised the research.
Toheed Iqbal: Reviewed and improved the article.

Conflict of interest
The authors have declared no conflict of interest.

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