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Field morphological identification of the most important acridid nymphs of cereal crops in Iran

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Abstract. The order Orthoptera, including locusts, grasshoppers, mole crickets, crickets and bushcrickets, are generally herbivores. With large-scale and single-crop agriculture development, some locusts and grasshoppers appeared as pests and sometimes caused famine and crisis. The plateau of Iran is the origin of many cereal cultivars. Since some morphological characters, particularly the male internal genitalia in nymphs do not differentiate enough for confident diagnosis; the classical morphological identification of grasshoppers is largely based on the characteristics of adults. However, identifying the nymphs is very important in the field from the economic point of view, because most crop loss is due to the nymphal damage. Although accurate molecular determination is possible even in the egg stage, this technique is costly and specialized. Thus, providing cheaper and simpler approaches have rational perspectives. Our studies showed that some morphological features in nymphs could be practised at a genus-level field identification, none of the important grasshoppers related to wheat cultivation in Iran has apterism, and only the macroscopic characteristics of nymphs of two ages before maturity provide the necessary accuracy for field identification. Therefore, from 2019 through 2021, morphological characteristics of developing organs that could be informative for taxonomy in the late-stage nymphs were investigated in 362 nymphs belonging to 22 genera of 8 subfamilies out of 2 families. As a result, some 35 most conservative characters and 79 character-states were extracted. Afterwards, an illustrated key for field genus identification was prepared based on a sort of data. Education and extension of these filed identification outputs can avoid wasting time and money and lessen pollution of the environment with hazardous chemical agricultural pesticides before any control operation.

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Introduction

Although some recent research dates limited early agriculture to 23 thousand years ago (Snir *et al.*, 2015), the Neolithic Revolution which formed approximately 10,000 B.C., might be the launch of the transition in human history. The gradual event revolved around small, travelling crowds of hunter-gatherers to large settlers in the Fertile Crescent. That event is the commencement of primary land-based civilization and cultivation of cereals (Abbo *et al.*, 2013). Shortly after, the Stone Age humans in other parts of the world also began to practice agriculture and locust problems emerged (Frahm & Carolus, 2022). Cereals were grown in the fertile crescent from Palestine and Lebanon to Syria, Iraq and Iran (Milić *et al.*, 2022) and many times in other fertile parts of the world, in polyphyletic domestication, progressively made the exploiter humans to identify insects and consequently their classification was noticed (Snir *et al.*, 2015). Almost all grasshoppers are herbivores. They

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quickly create massive swarms in suitable food and climatic conditions. Some species fly thousands of kilometres to reach green fields (Mishchenko, 1945).

One of the oldest Persian references to the outbreak of locusts is "Ershad al-Zeraeh", written by Qasim Ibn Yusuf Abu Nasri Heravi in 1515 AD. The author tried to provide solutions to combat locusts using some superstitions and supplications mainly through ancient Greek methods such as "locust water". It was believed that there was a water spring in Semirom (Isfahan Province, Iran) that attracted starlings, which were known to feed on locusts (Moshiri, 2011). An issue that became clear later is these energetic birds need water to drink and wash their beaks after killing and feeding on grasshoppers, so they fly to every spring and stream (Zomorrodi, 2003). In the contemporary era of Iran, the first report of a locust outbreak was in 1876 AD in the south of the country. However, local people and tourists reported some scattered outbreaks many years before that date, back to 1600 AD. Due to the outbreak of the desert locust *Schistocerca gregaria* (Forskål, 1775) in the early 20th century, the central government of Iran established an agricultural department in 1934, one of whose main tasks was to deal with locusts. In 1941, the invasion of locusts was very large and destroyed all the cotton crops around Varamin city in Tehran province (Zomorrodi, 2003).

The Orthoptera *sensu stricto* currently has a diversity of 29,442 species in the world with 8,124 species of the Tettigoniidae, 6,794 species of the Acrididae, 3,398 species of the Gryllidae and some smaller families (Bánki *et al.*, 2023). Among them, only a few species exist as pests (both common and general) (FAO, 2014), so approximately 500 species of this order can be considered pests and some 50 species can be classified as first-degree pests (Zhang *et al.*, 2019).

The Moroccan locust *Dociostaurus maroccanus* (Thunberg, 1815) on the slopes of the Alborz and Zagros Mountains causes the most damage, while in the central regions of Iran is rarely seen. Permanent desert locust breeding centres are mainly in Africa, Arabia, India, and Pakistan. The locust may transform under certain conditions from an "individual phase" to a "gregarious phase" and attack other regions, including Iran (Modarres-Awal, 1997). Almost 20 native short-horned grasshoppers and some bushcrickets attack cereal cultivation in Iran at the beginning of the cropping season (Keyhanian & Ghazavi, 2005). In the last few years, the outbreak of locusts, especially after the occurrence of monsoon rains and floods, has harmed the national economies of Iran, Pakistan and India (Riffat *et al.*, 2021).

Controlling the growing population of locusts, which are inherently invasive, is critical to food security around the world. On the other hand, the dispatch of population control teams usually involves a significant cost, and large amounts of chemical insecticides used have serious and dangerous side effects on the environment (Lockwood *et al.*, 2000).

This is even though the increase in the population of native grasshoppers, which is possible under suitable climatic conditions, does not have this necessity, and with the deterioration of suitable nutritional conditions, the population quickly vanished by insectivores. Therefore, continuous monitoring, investigation of climatic factors, and scientific identification of locusts and their analysis are very necessary. These preventive strategies will lead to a reduction in damage to products, and as a result, fewer side effects will be imposed on the environment (Zhang *et al.*, 2019).

To determine the best strategy for controlling agricultural pest locusts, it is necessary to identify them precisely as soon as possible at the beginning of the season, when they are still in the early stages of nymphal stages. The existing identification keys are largely based on the morphological characteristics of the adults. In this way, the pests are identified after sustaining the damage to the agricultural products. The nymphs can be identified through molecular techniques; however, it is quite expensive and limited to the gene bank data.

Growth and development

Generally, after the grasshopper embryo reaches full development in the egg, it is enclosed in a thin yellow cuticular layer as well as a thick and hard white cuticular layer. It is difficult to tear the white layer during hatching, but a few days before hatching, on the sides of the first abdominal segment of the embryo, an enzyme is secreted from a pair of small protrusions called *pleuropodia*. It leads to the dissolution of this white, thick and becomes hard (Viscuso & Sottile, 2008), the secretion of which is controlled by some hormones secreted from the prothoracic gland (Fig. 1) (Konopová *et al.*, 2020). The creature that emerges out of the egg is called a larva, which does not resemble a grasshopper in its larval movements.

The larvae are born at the right time, temperature and humidity for each grasshopper species, and this birth is usually the same for all embryos. The shape of this organism is slightly different from the fully developed embryo inside the egg. The head is bent down and the antenna and legs are close to the body. A thin transparent membrane that covers the whole body like a bag envelops the whole insect, but separate sleeves for the legs, the end of the antenna and the cerci are observable. This membrane, which is called provisional or serosal cuticle, in the past was considered the homologue of the amnion, which is now rejected (Konopová *et al.*, 2020) because this membrane is a cuticle cover. It is chitinous and has no cellular structure. Soon, with the pressure of the bump between the animal's head and chest (Cervical ampulla), the larval shell will open and an intermediate moult will occur. Therefore, the larva is the 1st nymphal age, but to avoid confusion in the numbering, it is distinguished from the later nymphs by the special name "larval stage" (Uvarov, 1966, 1977; Lecoq & Zhang, 2019).

As the larva moults, the 1st instar nymph emerges, which is very pale, and a hint of darker patterns and colours specific to each species gradually appears after a few minutes. Changes in growth and skin decorations can be seen at all ages of the male genitalia, but the changes in the extraneous parts and external sexual organs can often be examined only at the last age of male genitalia. In most species, the 1st instar is usually only 2 to 3 mm long. The full-grown 1st instar is smooth and apparently without wing pads, and after a few days of feeding, it grows and moults. The 2nd instar has a more elongated body, but the wing bud is still very small. As the heat increases and after a few days of feeding, the 2nd instar nymphs moult and then the 3rd instar nymphs appear with a slightly longer body and more visible wing buds. The 4th instar is larger and heavier and has small triangular wing buds that extend from the pronotum to the hind, on the first abdominal cord. The wing buds have parallel and prominent clear veins. In many species, the wing bud has punctate decorations at this stage and later. Abdominal appendages and body colouration can help to classify and set the identification key for nymphs. Overturning of wing buds occurs at this age. In this event, the lower wings are placed on the body with faster growth than the growth of the wing covers (Fig. 2). By the 5th instar, grasshoppers are almost developmentally complete, except for the wings, which lack a cross vein, but sexual maturity has not yet occurred, and the male internal reproductive organs, which are essential for species-level identification, have not undergone sclerotinous differentiation. The 5th instar moults for the last time in about a week and after feeding in warm conditions, the adult insect appears. The soft wings are then filled up with hemolymph and dry within a day (Luong-Skovmand & Balança, 1999).

The study of the development of nymphs of important locusts damaging to agricultural products has shown that the size, colour, arrangement and placement of incomplete wings or wing buds in pre-adult nymphs can be used to determine the age of nymphs (Riffat & Waga, 2010). With the same point of view and to determine the middle nymphal stages, indicators such as wing buds' growth and position on the body in the desert locust, Asian locust *Locusta migratoria* (Linnaeus), 1758, the Moroccan grasshopper *Dociostaurus maroccanus* (Thunberg, 1815), the white-fronted bushcricket *Decticus albifrons* (Fabricius, 1775) and the green bushcricket *Tettigonia viridissima* (Linnaeus, 1758) were investigated in the country (Ghazavi, 2015). The ageing is appropriate for the implementation of locust control operations and reducing cost control.

Materials and methods

The most important short-horned grasshoppers causing some damage to grains in Iran were selected based on recent Country Reports (Anonymous, 2022). In this way, specimens of last ages of 362 nymphs belonging to 22 genera out of 8 subfamilies and 2 families were examined (Table 1) from 2019 through 2021. The nymphs of different stages were classified and compared morphologically. The colour, shape and macroscopic decorations of the most conservative characters were recorded and clustered according to each stage. The purpose of this step was to review and prepare a list of morphological characters and character-states to discover the feasibility of grouping and setting a higher taxonomy identification key to the nymphs in the field condition. The entire quantitative and qualitative comparisons to determine diagnostic indicators and identification keys were produced on pre-identified specimens stored in the Hayk Mirzayans Insect Museum (HMIM) housed in the Iranian Research Institute of Plant Protection.

Measurements, imaging and drawings

The purpose of this work was the feasibility of checking the results by experts and semi-expert observers in field conditions. Accordingly, ordinary drawing chess paper was used for measuring and detailed observation of depressions, ridges and counting of spines recorded using a hand-held loop with a magnification of at least 10 times. A Konica Minolta DiMage Z1 camera was used for digital photography. The images were drawn by Genius-G-Pen® M712X light pen and the drawings were processed in Abobe Photoshop® CC 2019.

Characteristics and identification keys

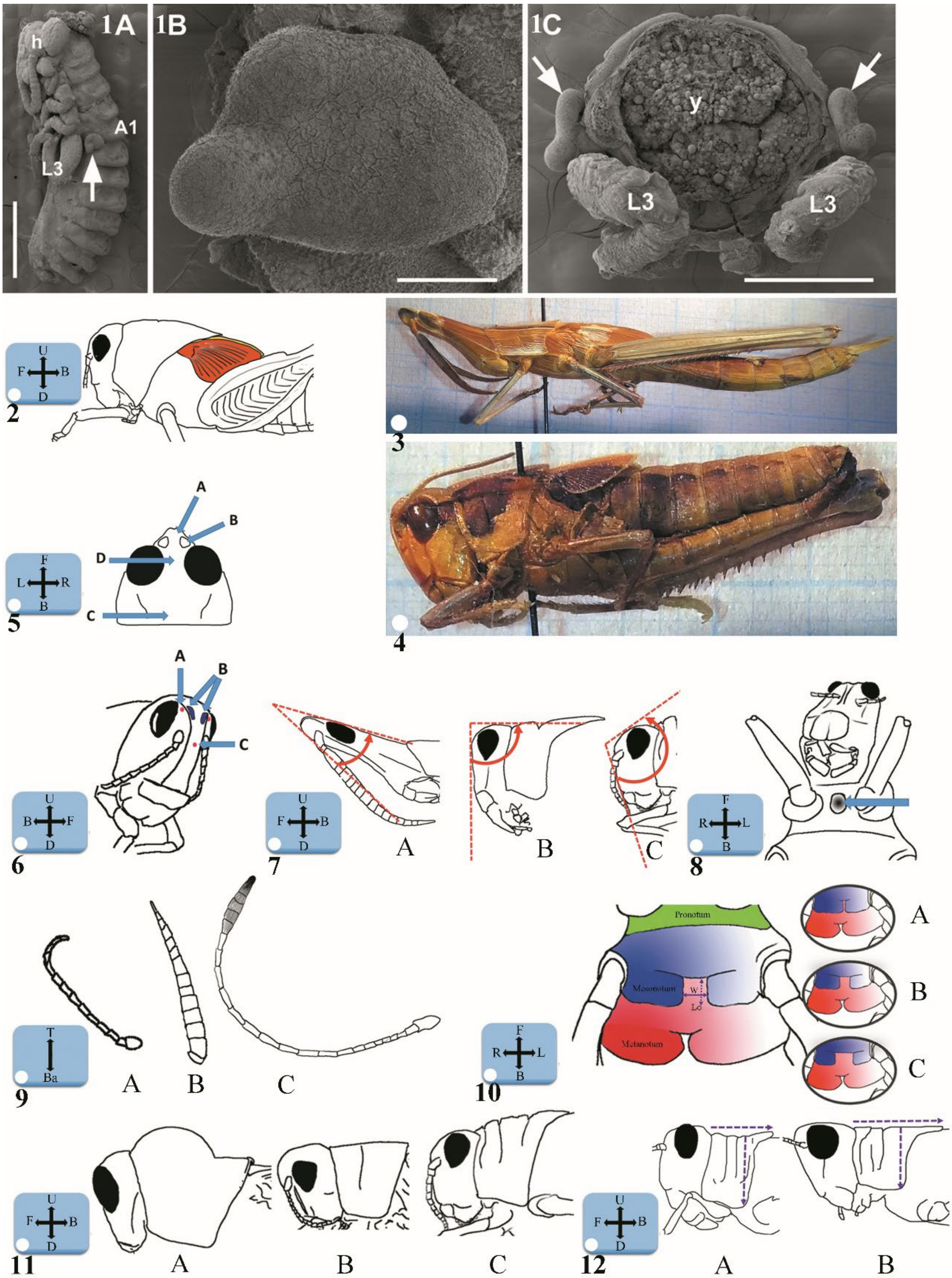
A list of characters and character-states prepared in the selected taxa passed through comparing the scientific description of them, examining the morphological identification keys of adult insects, as well as studying the preserved specimens and determining the type material preserved at HMIM. Then, the states observed in each characteristic in the nymphs of the last stages of the sorted acridids were tabulated and carefully reviewed. Subsequently, a traditional binary identification key developed *via* DKey software (Tofilski, 2018) referring to the illustrated information.

Results

The morphological features of the developing organs that can be used for the field classification and diagnosis in late-stage nymphs were investigated in 428 nymphs belonging to 31 genera from 16 subfamilies and 11 families in 4 orders (Table 1).

Table 1. The number of late instar acridid nymphs (Orthoptera) causing damage to cereals, which have some pre-adult morphological resemblances

Family	Subfamily	Genus	Male	Female	Agricultural importance (Iran condition)
Acrididae	Acridinae	*Truxalis* Fabricius, 1775	8	6	low
Acrididae	Acridinae	*Acrida* Linnaeus, 1758	11	13	intermediate
Acrididae	Acridinae	*Duroniella* Bolívar, 1908	4	5	low
Acrididae	Calliptaminae	*Calliptamus* Serville, 1831	9	7	high
Acrididae	Cyrtacanthacridinae	*Schistocerca* Stål, 1873	15	22	high
Acrididae	Cyrtacanthacridinae	*Anacridium* Uvarov, 1923	8	11	intermediate
Acrididae	Eyprepocnemidinae	*Heteracris* Walker, 1870	4	7	low
Acrididae	Gomphocerinae	*Gomphocerus* Thunberg, 1815	3	4	low
Acrididae	Gomphocerinae	*Ochrilidia* Stål, 1873	5	6	low
Acrididae	Gomphocerinae	*Dociostaurus* Fieber, 1853	22	28	high
Acrididae	Gomphocerinae	*Ramburiella* Bolívar, 1906	6	8	high
Acrididae	Gomphocerinae	*Euchorthippus* Tarbinsky, 1926	4	6	low
Acrididae	Gomphocerinae	*Chorthippus* Fieber, 1852	11	14	high
Acrididae	Oedipodinae	*Oedaleus* Fieber, 1853	7	5	intermediate
Acrididae	Oedipodinae	*Locusta* Linnaeus, 1758	11	10	high
Acrididae	Oedipodinae	*Oedipoda* Latreille, 1829	9	8	intermediate
Acrididae	Oedipodinae	*Pyrgodera* Fischer von Waldheim, 1846	6	5	low
Acrididae	Oedipodinae	*Acrotylus* Fieber, 1853	3	4	intermediate
Acrididae	Oedipodinae	*Sphingonotus* Fieber, 1852	6	4	intermediate
Acrididae	Oedipodinae	*Aiolopus* Fieber, 1853	11	12	high
Dericorythidae	Dericorythinae	*Dericorys* Serville, 1838	5	6	high
Dericorythidae	Iranellinae	*Iranella* Uvarov, 1922	2	1	low



Figs 1-12. 1. SEM images of fully developed pleuropodia (eight-days-old): A. Embryo; B. Enlarged image of the left pleuropodium; C. Transverse section of the embryo, pleuropodiums indicated by arrows. A1. The first abdominal segment; h. head; L3. Hind leg (third); y. Yolk. Scales: A. 1 millimeter; B. 100 μm; C. 500 μm (after Konopová *et al.*, 2020). 2. Reversal of wing buds, 4th instar of *Oedipoda* sp., hind wing buds (red) covers the body. 3. General shape of an elongated body, *Truxalis* sp. (millimeter grid paper as background). 4. General shape of a robust body, *Dociostaurus* sp. (millimeter grid paper as background). 5. Head of *Dericorys* sp., top view. A. Fasitgium, B. Foveola, C. Occiput, D. Vertex. 6. Head of *Dericorys* sp., side view. A. Lateral ocellus, B. Foveola, C. Middle ocellus. 7. The angle between the axes of the head and mouthparts: A. Acute, *Truxalis* sp.; B. Almost vertical, *Oedipoda* sp.; C. Obtuse angle, *Neoparanothrotes* sp. 8. Terga of thorax, prosternal process indicated by arrow, *Calliptamus* sp. 9. The antenna A. filiform, B, ensiform, C. capitate-clavate. 10. Pronotum transverse groove: A. Without, *Dericorys* sp.; B. With one groove, *Iranella* sp., *Oedaleus* sp.; C. With two or more grooves,

Oedipoda sp. 11. Meso- and meta-thoracic space: A. Narrow and elongated, *Schistocerca* sp.; B. More or less square, *Dericorys* sp.; C. Wider than long, *Iranella* sp. 12. Pronotum: A. The length is almost equal to the height, *Acrotylus* sp.; B. Longer than height, *Sphingonotus* sp. U: Up, D: Down, F: Front, B: Back, T: Tip, Ba: Base, R: Right, L: Left.

Our studies showed that some morphological features in nymphs could be practised at a genus-level field identification, none of the important grasshoppers related to wheat cultivation in Iran has apterism, and only the macroscopic characteristics of nymphs of two ages before maturity provide the necessary accuracy for field identification. Hence, the younger nymphs were left out.

It should be noted that in the 3rd instar (in grasshoppers that have 5 instars), some colour characteristics and the emergence of wing buds, as well as some external male and female sexual extravagances appear. Although the comparison of the macroscopic characteristics of the 3rd instar nymphs did not provide the necessary accuracy for field taxonomy and was excluded from the present research, if necessary, the following diagnostic key, which is not focused on the wing bud, is functional to an acceptable extent for the 3rd instar nymphs as well.

By comparing the scientific description of the species, examining the morphological identification keys of adult insects and finally separating and comparing the previously identified nymphs at the HMIM, a list of possible characters and character-states prepared an identification key to the genus level prepared.

Morphological identification key to the genera of Acrididae nymphs

1 With prosternal process (Fig. 8) pointed or raised (Catantopinae *sensu lato*).. 2

- Without a clear prosternal process 7

2 (1) The end of the hind tibia with a spine on the external upper side (Fig. 21A) 3

- The end of the hind tibia without spines on the external upper side (Fig. 21B)................................ 4

3 (2) Body elongated; head with distinct and clear foveola (Fig. 6B) near fastigium (Fig. 5A); sub-frontal groove of the head in the area of the middle ocellus with comparatively parallel edges, not compressed (Fig. 24A); the prozona prominent, the pronotum without any distinct transverse groove (Fig. 10A); the meso- and meta-thoracic space more or less square (Fig. 11B) (Dericorythinae) .... *Dericorys*

- Body robust; head without distinguishable foveola; sub-frontal groove of the head in the area of the middle ocellus with non-parallel edges, the sub-frontal groove compact (Fig. 24B); the prozona wrinkled and pointed, the pronotum with one distinct transverse groove (Fig. 10C); the sternal lobes of meso- and meta- thorax almost square or wider than long (Fig. 11C) (Iranellinae) .... *Iranella*

4 (2) The space between the sternal lobes of the meso- and meta- thorax narrow and elongated (Fig. 11A) (Cyrtacanthacridinae) 5

- The space between the sternal lobes of the meso- and meta- thorax wider than long (Fig. 11C)...................... 6

5 (4) The longitudinal-median carina of pronotum non-prominent (Fig. 14A); the male subgenital plate depressed in the middle and bilobed terminally (Fig. 25C) .... *Schistocerca*

- The longitudinal-median carina of the pronotum prominent (Fig. 14B); the male subgenital plate protruding in the middle and the sides with a depression trilobed terminally (Fig. 25D).. *Anacridium*

6 (4) Longitudinal lateral carina of pronotum distinct, leading to the hind edge of the metazona (Fig. 15A); the basal half top of the upper ovipositor valves convex (Fig. 22A); the cerci of the male nymphs with two lobes at the lower end (Fig. 26A); hind tibiae with 8 to 10 spines on the outer-upper side; hind femur stout and bulky (Fig. 18A) (Calliptaminae) .... *Calliptamus*

- Longitudinal lateral carina of pronotum in the metazona indistinct and disappearing to the hind edge (Fig. 15B); the basal half top of the upper ovipositor valves with sinusoidal curve (Fig. 22B); the cerci of the male nymphs without lobes at the end (Fig. 26B); hind tibiae with 14 to 16 spines on the outer-upper side; hind femur elongated and cylindrical (Fig. 18B); (Eyprepocnemidinae) .... *Heteracris*

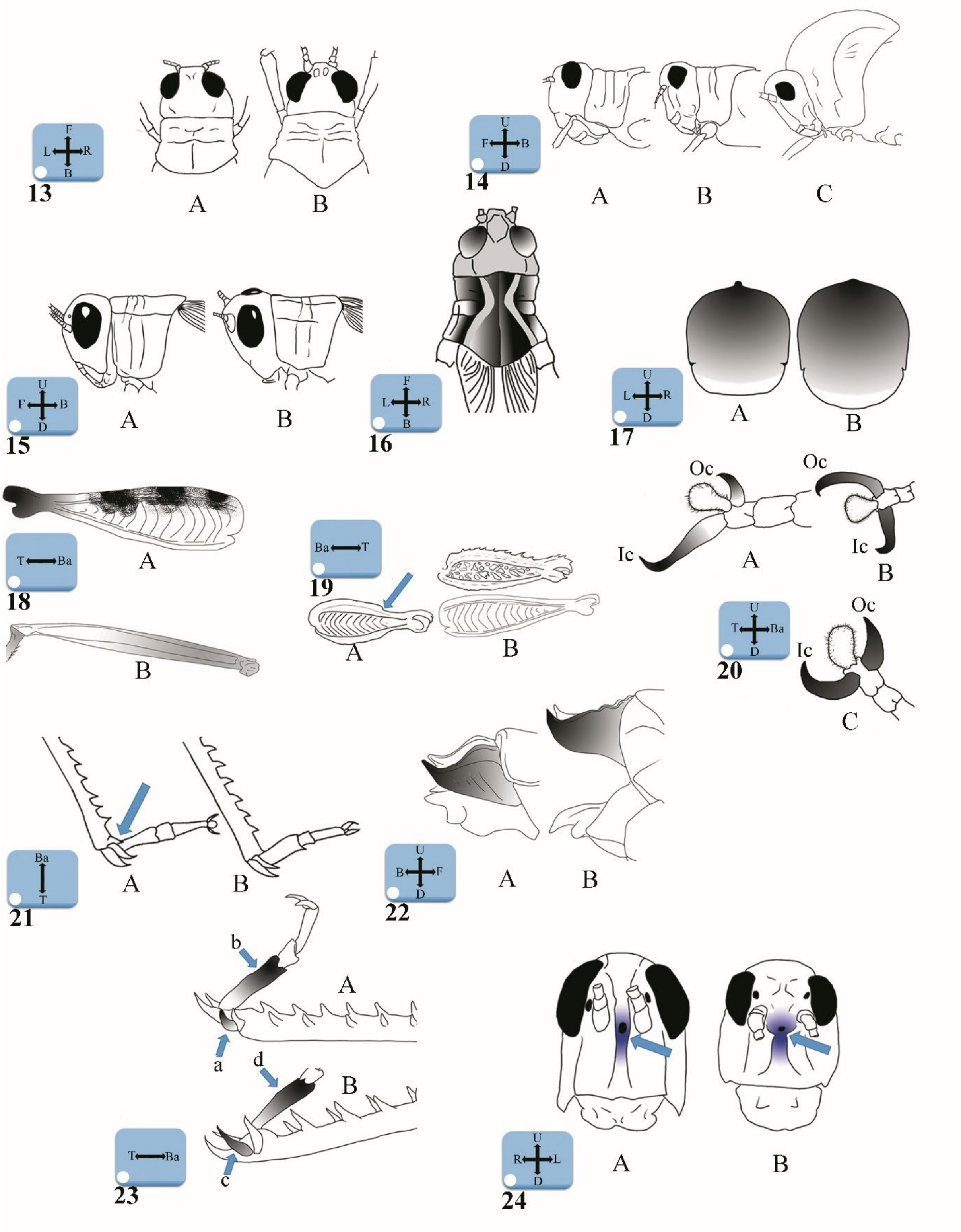
7 (1) Head strongly conical, the angle between the axes of the head and mouthparts acute, the mouthparts inclined towards the back (Figs 3 and 7A) (Acridinae).. 8

- The angle between the axes of the head and mouthparts is almost vertical (Acrididae) (Fig 4) or a little less vertical (Oedipodinae) (Fig. 7B) or obtuse (Pamphagidae) (Fig. 7C) 9

8 (7) Top mid of abdominal terga with a distinct longitudinal keel in vertical cross section (Fig. 17A) ..... *Truxalis*
- Top mid of abdominal terga without prominent longitudinal keel in vertical cross section (Fig. 17B) ...... *Acrida*
9 (7) The antenna filiform (Fig. 9A) 10
- The antenna is not ensiform (Fig. 9B), rarely capitate-clavate (particularly in males) (Fig. 9C) 17
10 (9) Pronotum with one transverse groove (Fig. 10B) or without it (Fig. 10A) 11
- Pronotum with at least two transverse grooves (Fig. 10C).. 12
11 (10) Pronotum without prominent and distinct longitudinal median carina, the shape of the longitudinal-lateral carina and the colouring of pronotum reminiscent of the letter X (Fig. 16).. *Oedaleus*
- Pronotum with prominent and distinct longitudinal median carina, without X mark ....*Locusta*
12 (10) Longitudinal-upper keel in the middle of the hind femur with a fracture (Fig. 19A)...............................
.... *Oedipoda*
- Longitudinal-upper keel in the middle of the hind femur without breakage (Fig. 19B) 13
13 (12) Pronotum longitudinal median carina strongly prominent (Fig. 14C) ....*Pyrgodera*
- Pronotum longitudinal median carina projection normal or none (Fig. 14A and 14B) 14
14 (13) Pronotum length almost equal to the height (Fig. 12A), the pronotum edge round with no clear angle at the end, more or less rounded posteriorly (Fig. 13B); legs hirsute ....*Acrotylus*
- Elongated pronotum with a hind edge in the middle grown towards the abdomen (Fig. 12B), the pronotum edge with a distinct angle posteriorly (Fig. 13A), legs without distinct hairs 15
15 (14) The lower spur at the end of the hind leg on the inner side, is short, less than half the length of the metatarsus (Fig. 23A) .... *Sphingonotus*
- The lower spur at the end of the hind leg on the inner side, elongated, equal to or more than half the length of the metatarsus (Fig. 23B) 16
16 (15) Pronotum without distinct longitudinal and lateral carina (Fig. 15B)...................................... *Aiolopus*
- Pronotum, at least in the prozona, with distinct longitudinal and lateral carina ....*Duroniella*
17 (9) The antenna capitate-clavate (especially in males) (Fig. 9C) ....*Gomphocerus*
- Antenna not swollen at the end 18
18 (17) Antenna ensiform (Fig. 9B)...*Ochrilidia*
- Antenna not ensiform... 19
19 (18) The shape and colouration of the longitudinal and lateral carina of the pronotum reminiscent of the letter X (Fig. 16) (Gomphocerinae)..*Dociostaurus*
- No distinct reminiscent of the letter X on the pronotum 20
20 (19) Longitudinal lateral carinae of the pronotum almost indistinct...*Ramburiella*
- Longitudinal lateral carinae of the pronotum developed, distinct, slightly fading in the metazona posteriorly . 21
21 (20) The inner claw of the middle and hind tarsa is longer than the outer claw, the outer claw in the front tarsus is longer than the inner claw (Fig. 20A and 20B); the surface of the thorax pleura wrinkled; the male subgenital plate acute posteriorly (Fig. 25B) .... *Euchorthippus*
- The inner and outer claws of tarsa are almost the same size (Fig. 20C); the surface of the thorax pleura is smooth; the male subgenital plate is truncated posteriorly (Fig. 25A) ....*Chorthippus*

Discussion

A review of the annual Country Reports on the most important pests in the last ten years shows several locusts and grasshoppers enlisted as cereal pests (Anonymous, 2022). In particular, at the beginning of the cropping season, most of the fields are not green yet, and therefore wheat, barley and alfalfa fields are temporary hosts for many species of hungry insect acridid nymphs, whilst the wheat farms have the highest biodiversity (Mahloji Rad, *et al.*, 2019). Particularly, with the increase of pasture greenness, large parts of these different species' nymphs leave the field. Accordingly, simply framing and counting the nymphs does not provide necessarily convincible statistical information to predict the "pest species" population. Therefore, the first step to controlling a true pest is to identify it correctly.



Figs 13-24. 13. The hind edge of the pronotum: A. Round, without a distinct angle, *Sphingonotus* sp.; B. Angled, *Acrotylus* sp. 14. Pronotum longitudinal median carina: A. Non-prominent, *Schistocerca* sp.; B. Prominent, *Anacridium* sp.; C. Strongly projecting, *Pyrgodera* sp. 15. Longitudinal lateral carina of pronotum: A. Distinct, leading to the hind edge of metazona, *Calliptamus* sp.; B. Indistinct and disappearing to the hind edge *Heteracris* sp. 16. The shape and colouration of the pronotum is reminiscent of the letter X. 17. Vertical cross section of the abdomen: A. Top mid of tergum with a distinct longitudinal keel, *Truxalis* sp.; B. Top mid of tergum without a distinct longitudinal keel, *Acrida* sp. 18. Hind femur: A. Stout and bulky; B. Elongated and cylindrical. 19. Longitudinal-upper keel in the middle of the hind femur: A. With a fracture, *Oedipoda* sp.; B. Without breakage. 20. Claws: A. The inner claw of the middle and hind tarsa longer than the outer claw; B. The outer claw of the

front tarsus longer than the inner claw, both *Euchorthippus* sp.; C. The inner and outer claws of all tarsa are almost the same size, *Chorthippus* sp. 21. The end of the hind tibia on the external side: A. With a spine on the upper side; B. Without spines on the upper side. 22. The basal half top of the upper ovipositor valves: A. Convex, *Calliptamus* sp.; B. Sinusoidal, *Heteracris* sp. 23. Hind leg, inner side: A. The lower spur (a) short, less than half the length of the metatarsus (b), *Sphingonotus* sp.; B. The lower spur (c) approximately half the length of metatarsus (d), *Duroniella* sp. 24. Head front view, subfrontal groove in the area of the middle ocellus: A. Comparatively parallel, not compressed, *Dericorys* sp.; B. Non-parallel and compact, *Iranella* sp. Oc: Outer claw, Ic: Inner claw, U: Up, D: Down, F: Front, B: Back, T: Tip, Ba: Base, R: Right, L: Left

Once its scientific name is known, it facilitates access to information about the damage it can cause and how to investigate and control it. Conversely, as with most insects, definitive morphological diagnosis of Orthoptera is mainly based on adult information.

This issue is common in other insect orders too. Within the available taxonomic publications, there are only a few keys to identify immature insects, which are commonly compiled for holometabolous insects (Chu, 1949).

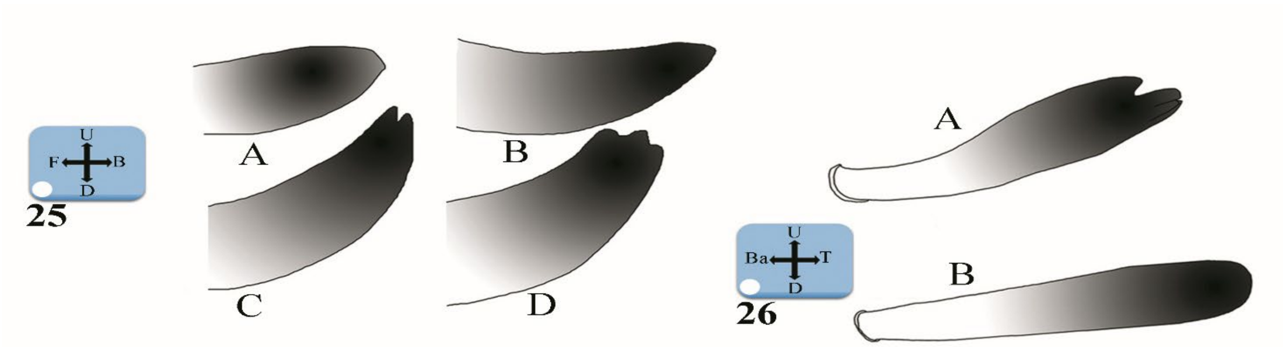
In insects with incomplete metamorphosis, nymphs have gradual development and the wing pattern and venation show changes in growth. Generally, a definitive diagnosis of adult insects is made using the characteristics of male (and sometimes female) sexual organs, which are not yet developed in immature insects (Bei-Bienko & Mishchenko, 1963; Bei-Bienko, 1967).

Although the embryonic and nymphal metamorphosis of grasshoppers has been carefully studied by some pioneers (Uvarov, 1966, 1977); however, the diagnosis of the pest nymphs of *Aiolopus thalassinus* (Fabricius) can be considered the first agricultural research (Baloch, 1978). In another study, some morphological differences between three species of short-horned grasshoppers from the genus *Hieroglyphus* Krauss were investigated (Riffat & Waga, 2010). A few years later, in a similar study, the nymphs of two species of short-horned grasshoppers belonging to the genus *Aiolopus* Fieber, which are among agricultural pests, were described and separated (Soomro *et al.*, 2015). The research continued later on the nymphs of *Sphingonotus* Fieber (Soomro *et al.*, 2019).

Economic and environmental outputs

The scientific identification of nymphs of damaging grasshoppers to wheat precedes the determination of their age. The main question is whether it is possible to determine the scientific identity of these insects by examining the most important indicators and morphological characteristics of locust nymphs before suffering damage, maturity and distance. Do locusts adopt the best control strategy?

The present work is a new experience in Iran. The main goal is to determine low-cost but accurate methods to determine the scientific identity of locust nymphs. It was shown that non-microscopic identification of nymphs could only be possible by comparing the last instars. The coincidence of the emergence of the last instars with the peak of damage shows the key role of the outputs of the present research.



Figs 25-26. 25. Male subgenital plate posteriorly: A. Truncated, *Chorthippus* sp.; B. Acute, *Euchorthippus* sp.; C. Depressed in the middle and bilobed, *Schistocerca* sp.; D. Protruding in the middle and the sides with a depression (three lobes), *Anacridium* sp. 26. Male cercus: A. With two lobes at the end on the lower side, *Calliptamus* sp.; B. Broad, round, curved downward at the end and without lobes, *Heteracris* sp. Up, D: Down, F: Front, B: Back, T: Tip, Ba: Base.

The crop loss, because of locusts, in the 1st and 2nd ages are insignificant. The nymphs of the 3rd age need much more nutrition. At the same time, along with the phenology of wheat growth, accuracy is necessary for the scientific diagnosis of the pest. However, the feeding activity of the nymphs reaches the highest in the 4th age. The 5th instars feed almost all hours of the day and night (if the plant is available) (Johnson, 2010).

The most important output of the present work is the negligent diagnosis when framing and counting the nymphs of the last age of locusts, which are suspected to be, for instance, the Moroccan Locust (merely because of the X mark on the pronotum). This case often happens in some habitats and causes wrong reports by experts and observers, the waste of energy and funds for the operation of the focus and the extensive and dangerous chemical interference in the biological diversity of fields and pastures. Meanwhile, several species belonging to some genera of short-horned grasshoppers own the X mark with some minor differences, which might confuse some field operators. Therefore, adopting scientific methods that can train experts and observers in determining a more accurate scientific identity of the classification of locust nymphs can have direct short-term and indirect long-term economic benefits in improving locust outbreak control operations, saving biodiversity and increasing human safety.

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تشخیص میدانی مهمترین ملخ‌های شاخک‌کوتاه زراعت غلات ایران بر مبنای شکل‌شناسی پوره‌ها

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چکیده

راست‌بالان شامل ملخ‌های بومی و مهاجر شاخک‌کوتاه و شاخک‌بلند، جیرجیرک‌ها و آبدزدک‌ها عموماً حشراتی گیاهخوار هستند. با توسعهٔ کشاورزی کلان و تک زراعی، برخی از ملخ‌های بومی و مهاجر شاخک‌کوتاه به صورت آفت باعث قحطی و بحران شده‌اند. فلات ایران خاستگاه بسیاری از ارقام غلات است. از آنجایی که برخی از ویژگی‌های شکل‌شناسی، به ویژه اندام زادآوری داخلی نر، هنوز در پوره‌ها به اندازهٔ کافی برای تشخیص و تفکیک علمی متمایز نشده‌اند، عمدتاً شناسایی شکل‌شناسی ملخ‌ها بر ویژگی‌های حشرات بالغ استوار است. در عین حال، شناسایی پوره‌ها در شرایط مزرعه از نظر اقتصادی بسیار حائز اهمیت است، زیرا آن‌ها بیشترین خسارت را به محصول وارد می‌کنند. تشخیص دقیق هویت علمی گونه‌ها به روش مولکولی، حتی در مرحلهٔ تخم نیز امکان‌پذیر است، اما این تکنیک بسیار گران و تخصصی است. بنابراین ارائهٔ روش‌هایی ارزان‌تر و ساده‌تر، عقلانی به نظر می‌رسد. بررسی‌های ما نشان داد که اولاً در شناسایی صحرایی، از برخی ویژگی‌های شکل‌شناسی پوره‌ها می‌توان در سطح جنس استفاده کرد، ثانیاً هیچ یک از ملخ‌های شاخک‌کوتاه مهم در زراعت گندم در ایران حالت بی‌بالی ندارند و ثالثاً ویژگی‌های شکل‌شناسی ماکروسکوپی پوره‌های دو سن قبل از بلوغ، دقت لازم برای این سطح از تشخیص صحرایی را فراهم می‌آورند. بنابراین، طی سال‌های ۱۳۹۸ تا ۱۴۰۱ خورشیدی، ویژگی‌های شکل‌شناسی اندام‌هایی که می‌توانستند برای رده‌بندی پوره‌های قبل از بلوغ مفید و پایدار باشند، در ۳۶۲ پوره متعلق به ۲۲ جنس از ۸ زیرخانواده و ۲ خانواده بررسی شدند. در نتیجه ۳۵ ویژگی شکل‌شناسی ثابت‌تر و ۷۹ حالت ویژگی استخراج شد. سپس بر اساس گزیده‌ای از این ویژگی‌ها، کلید مصوری برای شناسایی صحرایی پوره‌ها در سطح جنس تهیه شد. آموزش و گسترش بروندادهای تحقیق حاضر می‌تواند قبل از هر گونه عملیات کنترل، مانع اتلاف وقت و هزینه و همچنین آلودگی محیط زیست با سموم شیمیایی خطرناک کشاورزی شود.

کلمات کلیدی: تشخیص مزرعه‌ای، کلید شناسایی، نابالغ، ملخ شاخک‌کوتاه، رده‌بندی

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