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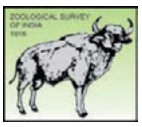

Preliminary observation on the polymorphic forms in three species of the Genus *Lepidocephalichthys* Bleeker, 1863 of Northeast India

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Abstract

The spined loaches under the genus *Lepidocephalichthys* Bleeker, 1863 under family Cobitidae are colourful freshwater fishes with both ornamental and food values and are characterized by the presence of a sub-orbital spine and pectoral rod formed by the fused last two pectoral-fin rays in males. Out of the 9 valid species of *Lepidocephalichthys* in the Northeateastern India, three species viz. *L. berdmorei, L. guntea,* and *L. irrorata* show great polymorphic features in terms of body morphology, meristic data and colour. In view of this, the current preliminary study has been taken up to contribute in solving the existing taxonomic ambiguities by documenting the various polymorphic forms observed in *Lepidocephalichthys* of the Northeastern India. Multiple comparisons through morphometry, meristic, anatomy and osteology of the specimens collected from different river systems from different parts of the Northeast India has been employed. The present study is based on the observation of 127 specimens of *Lepidocephalichthys* collected from various region of the Northeastern India and it has revealed the presence of polymorphism in three species viz. *Lepidocephalichthys berdmorei, L. guntea* and *L. irrorata*. *Lepidocephalichthys berdmorei* has been registered with 6 polymorphs, *L. guntea* with 5 and *L. irrorata* with 2. Further understanding on their molecular characterization is suggested to confirm the taxonomic status of these polymorphs.

Keywords: Spined loaches, *Lepidocephalichthys*, Cobitidae, sub-orbital spine, polymorphism, Northeastern India

Introduction

Polymorphism can be defined as the occurrence of two or more clearly different phenotypes or forms within the same population of a species. This may be a result of the occurrence of intraspecific morphs within a single population exhibiting different niche use known as the resource polymorphism. It is wide spread over several taxa including fish, amphibians and birds and may play important roles in population divergence and speciation (Smith & Skúlason, 1996). This phenomenon can be due to the occurrence of genetic differences, phenotypic plasticity, or a combination of both (Komiya *et al.,* 2011). Phenotypic plasticity can be defined as the ability of individual genotypes to produce different phenotypes when exposed to different environmental conditions (Pigliucci *et al.,* 2006). The phenomenon of polymorphism often creates confusion in the field of taxonomy leading to certain taxonomic ambiguities demanding proper studies.

Lepidocephalichthys are the freshwater loaches belonging to the family Cobitidae which is known as the family of spined loaches characterized by the presence of three pairs of barbells, two rostral pair and one maxillary pair; a pair of flaps on lower lip, each flap with thickened inner fold ending in small, barbel-like projection; a spine below the eye and 7th and 8th rays of the pectoral fin modified and fused with or without flange known as the Pectoral rod in males (Havird & Page, 2010; Kottelat, 2017). They are widely distributed in Asia, from India to China, Vietnam, Laos, and Cambodia and south to Borneo and Java (Kottelat & Lim, 1992; Menon, 1992; Arunkumar, 2000). A total of 19 valid species are known under genus *Lepidocephalichthys* globally

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(Fricke *et al.,* 2023), out of which 9 species are found in the Northeastern India viz., *Lepidocephalichthys alkaia* Havird & Page, 2010, *L. annandalei* Chaudhuri, 1912, *L. arunachalensis* (Datta & Barman, 1984), *L. berdmorei* (Blyth, 1860), *L. goalparensis* Pillai & Yazdani, 1976, *L. guntea* (Hamilton, 1822), *L. irrorata* Hora, 1921, *L. longipinnis* (Menon, 1992) and *L. micropogon* (Blyth, 1860). The fishes under this genus are facing taxonomic ambiguities as some of the species shows different pattern of polymorphism. There have been no studies done till date on polymorphism of the species under this genus. However, Vishwanath (2021) reported the presence of different phenotypic features in different specimens of *Lepidocephalichthys berdmorei* and *L. guntea* in the Northeastern India. Looking at recently collected *Lepidocephalichthys* species and those specimens deposited in the Assam Don Bosco University Museum of Fish we have found variations in morphology particularly in colour patterns within three species viz. *Lepidocephalichthys berdmorei, L. guntea* and *L. irrorata*. To understand their taxonomic identity and confirmation of each species, the present study has been taken up to document a preliminary observation of the polymorphic forms in these three species of the Northeastern India.

Materials and methods

Multiple comparisons of the polymorphic specimens through morphometry, meristic data, anatomy and osteology have been employed. Counts and measurements follow Kottelat (1990) and Kottelat & Freyhof (2007). Point to point measurements were made on the left side of specimens whenever possible, using dial calipers (Freemans FVD200 0-200 mm) to the nearest 0.1 mm. Fin rays were counted and scales were observed using Magnus stereo zoom microscope under transmitted and reflected light. Clearing and staining of bones followed Taylor & Van Dyke (1985) with slight modifications. Identification of bones is based on Sawada (1982) and Prokofiev (2009, 2010). The morphometry, meristic counts along with total vertebrae number, the position of dorsal fin insertion, the position of anal fin insertion, caudal complex and neurocranium structure were taken into account to group or classify as same species showing polymorphism. Variations on the number, size and pattern of blotches on lateral surface, bars on dorsum, presence and absence of stripe, presence and absence of dark patches are some of the characters seen in different samples of *L. berdmorei, L. guntea* and *L. irrorata*. The variations here are interpreted as intraspecific polymorphism. The polymorphic forms reported here are different from the sexual dimorphic forms as these forms were observed both in males and females. The study has been carried out based on the species deposited in Assam Don Bosco University Museum of Fish (ADBU-MF) Assam, India, collected from various river systems of Northeastern India. The numbers written in the parenthesis in the osteological features of Table 2 represents the frequency of observation.

Abbreviations used: ADBU-MF, Assam Don Bosco Museum of Fish, Assam, India; SL, Standard Length.

Materials examined

Lepidocephalichthys berdmorei: ADBU-MF/5019/1/i-x, 10 exs., 55.5–82.0 mm SL; India: Manipur, Bishnupur district, Nambol bazar (Chindwin drainage system); K. Velentina, 17.x.2021. ADBU-MF/5019/4/i-ix, 9 exs., 59.0–71.7 mm SL; India: Manipur: Moreh, (Chindwin drainage system); K. Velentina, 13.xi.2021. ADBU-MF/5019/4/x-xi, 2 exs., 54.2–72.4 mm SL; India: Nagaland, Zunheboto, Tizu River (Chindwin drainage system); Nilibo A., 05.viii.2022. ADBU-MF/5019/3/i, 67.5 mm SL; India: Manipur: Bishnupur district, Nambol bazar (Chindwin drainage system); K. Velentina, 17.x.2021. ADBU-MF/5019/3/ii, 76.3 mm SL; India: Manipur: Chandel district, Moreh (Chindwin drainage system); K. Velentina, 13.xi.2021. ADBU-MF/5019/5/i-v, 5 exs., 55.5–73.6 mm SL; India: Manipur, Bishnupur district, Nambol bazar (Chindwin drainage system); K. Velentina, 17.x.2021. ADBU-MF/5019/5/vi-vii, 2 exs., 59.6–60.0 mm SL; India: Manipur, Bishnupur district, Moreh (Chindwin drainage system); K. Velentina, 17.x.2021. ADBU-MF/5019/5/viii, 72.4 mm SL; India: Nagaland, Zunheboto, Tizu River (Chindwin drainage system); Nilibo A., 04.iv.2022. ADBU-MF/5019/6/i-xii, 12 exs., 60.3–74.0 mm SL; India: Manipur, Chandel district, Moreh (Chindwin drainage system); K. Velentina, 13.xi.2021 & 05.viii.2022. ADBU-MF/5019/6/xiii-xiv, 2 exs., 54.8–77.1 mm SL; India: Nagaland, Zunheboto, Tizu River (Chindwin drainage system); Nilibo A., 04.iv.2022. ADBU-MF/5019/2/i-iv, 4 exs., 54.8–84.4 mm SL; India: Nagaland, Zunheboto, Tizu River (Chindwin drainage system); Nilibo A., 04.iv.2022 & 05.viii.2022.

Lepidocephalichthys guntea: ADBU-MF 5021/1/i-ix, 9 exs., 46.8–60.8 mm SL; India: Assam, Niz Jhaprabari, Assam, Udalguri district, Bhorla river (Brahmaputra drainage system); Sushmita M., 15.x.2021 & 07.x.2022. ADBU-MF 5021/1/x-xii, 3 exs., 46.4–53.4 mm SL; India: Meghalaya,

North Garo Hills, Rajasimla, Dudhnoi River, Rongkil stream (Brahmaputra drainage system); Wimarithy K. Marak, 24.ix.2022. ADBU-MF 5021/1/xii-xxi, 9 exs., 38.2–61.4 mm SL; India: Meghalaya, North Garo Hills, Resu, Damring River, Chidrang stream (Brahmaputra drainage system); Wimarithy & party, 05.x.2021 & 22.iii.2022. ADBU-MF 5021/2/i-ix, 9 exs., 39.4–48.1 mm SL; India: Meghalaya, North Garo Hills, Resu, Damring River, Chidrang stream (Brahmaputra drainage system); Wimarithy & party, 05.x.2021 & 22.iii.2022. ADBU-MF 5021/2/x-ix, 10 exs., 46.0–57.4 mm SL; India: Meghalaya, Chidrang stream (Brahmaputra drainage system); Wimarithy A Marak, 07.x.2022. ADBU-MF 5021/3/i-ii, 2 exs., 61.0–65.9 mm SL; Assam, Chirang district, Khujia river (Brahmaputra drainage system); Bidangshri B., 15.x.2021. ADBU-MF 5021/3/iii-xv, 13 exs., 40.0–56.5 mm SL; India: India: Meghalaya, North Garo Hills, Resu, Damring River, Chidrang stream (Brahmaputra drainage system); Wimarithy & Party, 05.x.2021 & 07.x.2022. ADBU-MF 5021/3/xvi-xix, 4 exs., 38.4–49.2 mm SL; India: Meghalaya, North Garo Hills, Rajasimla, Dudhnoi River, Rongkil stream (Brahmaputra drainage system) Wimarihthy K. Marak; 24.ix.2022. ADBU-MF 5021/3/ xx, 46.4 mm SL; India: Meghalaya, West Garo Hills, Edenbari, Ganol River (Barak–Surma–Meghna drainage system); Rikkambe, 24.ix.2022. ADBU-MF 5021/4/i, 42.0 mm SL; India: Assam, Chirang district, Khujia River (Brahmaputra drainage system); Bidangshri B., 22.iii.2022. ADBU-MF 5021/4/ii-iv, 3 exs., 47.4–56.2 mm SL; India: Meghalaya, North Garo Hills, Resu., Damring River, Chidrang stream (Brahmaputra drainage system); Wimarithy & Party, 22.iii.2022. ADBU-MF 5021/5/i-ix, 9 exs., 33.0–43.5 mm SL; India: Meghalaya, North Garo Hills, Rajasimla, Dudhnoi River, Rongkil stream (Brahmaputra drainage system); Wimarithy K. Marak, 24.ix.2022.

Lepidocephalichthys irrorata: ADBU-MF/5020/1/i-vi, 6 exs., 32.1–34.9 mm SL; ADBU-MF/5020/2/i-v, 5 exs., 26.0–37.0 mm SL; India: Manipur, Bishnupur district, Moirang, Loktak lake (Chindwin drainage system); K. Velentina, 03.iii.2022.

Results and discussion

The present study documented the phenomenon of polymorphism in three lepidocephalid loaches out of the total 9 occurring in the Northeastern India viz., *Lepidocephalichthys berdmorei*, *L. guntea* and *L. irrorata* as mentioned in Table 1. The meristic and the osteological data of these three species showing the phenomenon of polymorphism on the basis of which they have been grouped as same species even though they show various morphological differences Table 2. The list of the polymorphic characters observed in *Lepidocephalichthys berdmorei, L. guntea* and *L. irrorata* respectively are shown in Table 3–5.

Polymorphs in *Lepidocephalichthys berdmorei.* On the examination of 49 specimens of *Lepidocephalichthys berdmorei* collected from various part of the Chindwin drainage system in the Northeastern India, revealed six polymorphic forms (Table 2 & Figure 2 A –F). The first group of species comprises 10 specimens and is characterized with 6–14 dark brown blotches on the flank along the horizontal myosepta superimposing above light grey stripe (Figure 2A–i & ii). The second group of species includes 11 specimens and is characterized in having smaller spots on the flank along horizontal myosepta superimposing above a thin light grey stripe and small irregular spots on flank above and below the midlateral line (Figure 2B). The third group of species includes 2 specimens having a unique features like the absence of blotches or spots however a distinct stripe on flank along horizontal myosepta and the presence of irregular interconnected reticulated stripe and spots on dorsolateral above lateral line (Figure 2C). The fourth group of specimens are with regularly arranged dark brown saddles on dorsum represented by 8 specimens (Figure 2D) but absent in many of them. The fifth group of specimens are without bars on dorsum, just heavily speckled with dark spots and are represented by 14 specimens (Figure 2E). The sixth polymorph of *Lepidocephalithys berdmorei* is represented by 4 specimens showing the presence of a dark stripe along the midline of caudal fin (Figure 2F). The sixth polymorph of *Lepidocephalichthys berdmorei* has the appearance of *L. alkaia* however differ from it in the absence of dark bars on dorsum (vs. presence of numerous bars; 8–10 predorsal, 8–10 postdorsal). No major variations have been observed in the meristic and osteological data of these 6 polymorphs of *Lepidocephalichthys berdmoeri* (Table 2). Thus, a preliminary confirmation of six polymorphic forms of *L. berdmorei* have been found in the Northeastern India however a more elaborate study with their molecular data is needed for further understanding of this species group and to provide a proper taxonomic distinction of it from *Lepidocephalichthys alkaia*.

Polymorphs in *Lepidocephalichthys guntea.* On the examination of 73 specimens of *Lepidocephalichthys guntea* collected from various part of the Northeastern India

revealed the presence of five different polymorphs (Table 4; Figure 3 A–E). The first polymorph is represented by 21 specimens and is characterized by the presence of a broad dark stripe along horizontal myosepta on the flank appearing the superimposition of closely arranged blotches on dark background stripe and irregularly arranged reticulated dark marking on dorsum and dorsolateral portion of flank leaving a wide space without marking just above midlateral stripe (Figure 3A–i & ii). This form is common in males; however, it has also been observed in females. The second polymorph was registered from 19 specimens and can be distinguished in having a series of dots or spots along the horizontal midlateral stripe (Figure 3B–i & ii). The third polymorph was registered form 20 specimens and can be characterized by the presences of blotches on the horizontal myosepta of the flank with various irregularly arranged reticulated patches above or below the midlateral stripe (Figure 3C i–iii). This group again shows differences in the appearance according to the blotch number and the pattern of patches present. The fourth group polymorph, consists of 4 specimens, is characterized by having various dark patches on the body, some forming blotches but not necessarily all (Figure 3D). The fifth groups of polymorph have been observed from 9 specimens appears to be juvenile and is characterized in having a series of dots on along the horizontal myosepta of the flank with no blotches or dark patches (Figure 3E). This form is concluded to be a juvenile form based on the larger fin length as compared to the standard length.

Polymorphs in *Lepidocephalichthys irrorata*. The examination of 11 specimens of *Lepidocephalichthys irrorata* has revealed the presence of two polymorphic forms. The first group of polymorph was registered from 6 specimens and characterized by the presence of pale yellowish-white body with very light brown fainted spots (Figure 4A). The second polymorph was registered from 5 specimens and characterised with pale-olivaceous body speckled with numerous dark spots (Figure 4B). Moreover, it is known to have 2 rostral pair of barbels and one maxillary pair like all the other *Lepidocephalichthys*. The examination on the recent collection of *Lepidocephalichthys irrorata* from the same type locality showed the differences in the barbel numbers and structure in some group of specimens. However, due to lack of molecular and biological work as well as limited availability of samples, we could not conclude the identity of these two forms as polymorphic or a different species.

The polymorphic forms of *Lepidocephalichthys berdmorei*, *L. guntea* and *L. irrorata* are grouped separately based on the osteological data including the vertebrae count, fin insertions, neurocranium structure and caudal complex. The morphometric data and meristic data as well as the distinct diagnostic characters are also taken into account. Multiple comparisons and studies of the specimens collected from different river systems of Northeast India in different seasons have been carried out to conclude the existence of these polymorphic forms. The reasons for the existence of these polymorphic forms might be due to differences in food habits, the habitat including the environmental factors such as water quality, or may also be an outcome of the adaptation strategies to the changing environment. However, the current study carried out cannot come up to a conclusion as to why these polymorphic forms occur in this genus. This paper mainly aims on documenting the existence of these polymorphic forms. Therefore, additional works on molecular level as well as biological studies including habitat analysis are required in order to give them a proper taxonomic distinction as polymorphic forms which will eventually help in describing new taxa under the genus *Lepidocephalichthys*.

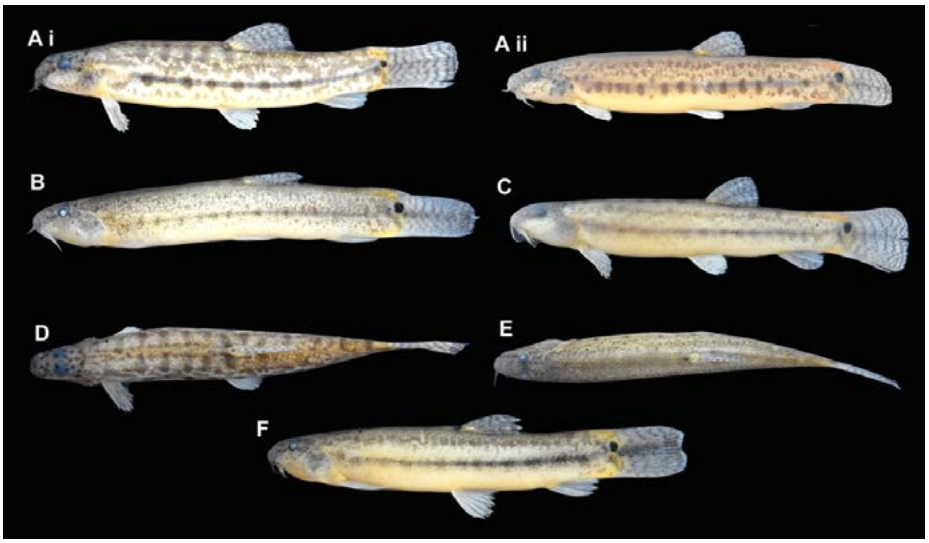

Figure 1. Polymorphic forms of *Lepidocephalichthys berdmorei* **(A i)** ADBU-MF 5019/1/i, 55.5 mm SL, lateral view; **(A ii)** ADBU-MF 5019/1/ii, 69.8 mm SL, lateral view; **(B)** ADBU-MF 5019/4, 60.5 mm SL, lateral view; **(C)** ADBU-MF 5019/3, 66.4 mm SL, lateral view; **(D)** ADBU-MF 5019/5, 55.5 mm SL, dorsal view; **(E)** ADBU-MF 5019/6, 63.12 mm SL, dorsal view; **(F)** ADBU-MF 5019/2, 63.3 mm SL, lateral view.

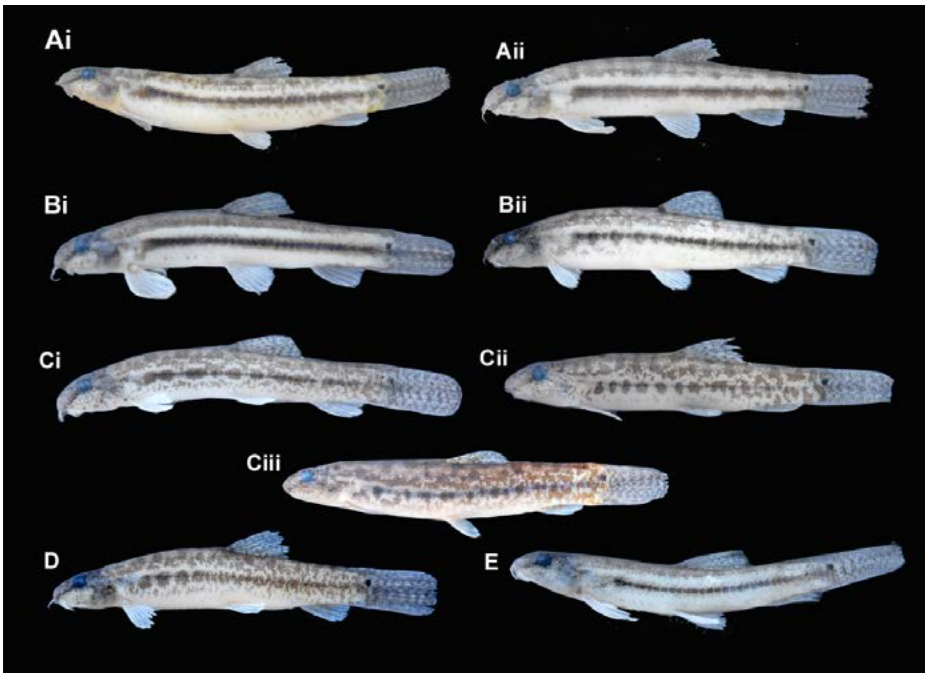

Figure 2. Polymorphic forms of *Lepidocephalichthys guntea* **(A i)** ADBU-MF 5021/1/i, 60.0 mm SL, lateral view; **(A ii)** ADBU-MF 5021/1/ii, 51.0 mm SL, lateral view; **(B i)** ADBU-MF 5021/2/i, 56.2 mm SL, lateral view; **(B ii)** ADBU-MF 5021/2/ii, 50.6 mm SL, lateral view; **(C i)** ADBU-MF 5021/3/i, 53.1 mm SL, lateral view; **(C ii)** ADBU-MF 5021/3/ii, 41.5 mm SL, lateral view; **(C iii)** ADBU-MF 5021/3/iii, 65.0 mm SL, lateral view; **(D)** ADBU-MF 5021/4, 50.3 mm SL, lateral view; **(E)** ADBU-MF 5021/5, 40.6 mm SL, lateral view.

Figure 3. Polymorphic forms of *Lepidocephalichthys irrorata* **(A)** ADBU-MF 5020/1, 28.9 mm SL, lateral view; (B) ADBU-MF 5020/2, 29.0 mm SL, lateral view.

Table 1. Lepidocephalid loaches under genus *Lepidoephalichthys* of Northeast India exhibiting polymorphism and their distribution. **BAR-SUR-MEGH —** Barak-Surma-Meghna drainage system, **CHIN** — Chindwin drainage system, **BRAH** — Brahmaputra drainage system, **'+'** indicates presence, **' '** indicates absence.

	BAR-SUR-MEGH	CHIN	BRAH	Polymorphism visible
Genus *Lepidocephalichthys* Bleeker, 1863				
1. L. alkaia Havird & Page, 2010	–	+	–	–
2. L. berdmorei (Blyth, 1860)	+	+	–	+
3. L. guntea (Hamilton, 1822)	+	–	+	+
4. L. irrorata Hora, 1921	–	+	–	+
5. *L. arunachalensis* (Datta & Barman, 1984)	–	–	+	–
6. L. goalparaensis Pillai & Yazdani, 1976	–	–	+	–

7. *L. annandalei* Chaudhuri, 1912	–	–	+	–
8. *L. longipinnis* (Menon, 1992)	–	–	+	–
9. *L. micropogon* (Blyth, 1860)	–	+	–	–

Table 2. Meristic and osteological data of three *Lepidocephalichthys* from the Northeastern India showing polymorphism viz; *Lepidocephalichthys berdmorei, L. guntea* and *L. irrorata.*

	L. berdmorei ADBU-MF/5019/1-6 N=6	*L. guntea* ADBU-MF/5021/1-9 N=9	*L. irrorata* ADBU-MF/5020/1-2 N=2
Meristic data			
Dorsal fin counts	3/6½	3/6½–7½	2/7
Pectoral fin counts	1/7	1/7–8	1/6
Pelvic fin counts	1/6–7	1/5–6	1/6
Anal fin counts	2–3/5½	3/5½	2/5
Caudal fin counts	14–15	14–15	14
Osteological data			
Total vertebrae	38(2), 39 (2), 40(2)	35(2), 36(5), 37(2)	39(2)
Abdominal vertebrae	19(3)–20(3)	17(1), 18(6), 19(2)	26(2)
Caudal vertebrae	18(2), 19(2), 20(2)	16(1), 18(7), 19(1)	13(2)
Predorsal vertebrae	15(2)–16(4)	12(1), 13(7), 14(1)	13(2)
Dorsal-fin insertion	15–16(2),16–17(4)	12–13(1), 13–14(7), 14–15(1)	13–14(2)
Anal-fin insertion	27–28(3), 28–29(3)	24–25 (5), 25–26(3), 26–27(1)	22–23(2)

Table 3. List of the polymorphic characters observed in *Lepidocephalichthys berdmorei.*

Sl. No.	Polymorphic character
1	Distinct dark brown blotches on flank along horizontal myosepta superimposing above light grey stripe, blotches varying in no. (6-14), various irregular patches on dorsolateral portion above mid- lateral line (Figure 2A–i & ii).
2	Smaller size spots on flank along horizontal myosepta superimposing above very thin light grey stripe, small irregular spots on flank above and below mid- lateral line (Figure 2B).
3	No distinct blotches but a distinct stripe on flank along horizontal myosepta, irregular interconnected reticulated strips and spots on dorsolateral above horizontal myosepta (Figure 2C).
4	Regularly arranged olivaceous dark brown saddles on dorsum (Figure 2D).
5	No saddles on dorsum, just mottled with dark spots (Figure 2E).
6	A dark stripe along the midline of caudal fin (Figure 2F).

Table 4. List of the polymorphic characters observed in *Lepidocephalichthys guntea.*

Sl. No.	Polymorphic character
1	Appearance as a broad dark stripe along horizontal myosepta, appearing the superimposition of closely arranged blotches on dark background stripe. Irregularly arranged reticulated dark marking on dorsum and dorso lateral portion of flank leaving a wide space without any marking just above midlateral stripe (Fig, 3A i & ii).
2	With series of dots or spots along the horizontal midlateral stripe (Figure 3B–i & ii).
3	Blotches on lateral surface with various patches (Figure 3C i–iii).
4	Various dark patches on the body some forming blotches (Figure 3D).
5.	Series of dots on the mid-lateral surface (juvenile) (Figure 3E).

Table 5. List of the polymorphic characters observed in *Lepidocephalichthys irrorata.*

Sl. No.	Polymorphic character
1.	Pale yellowish-white body with very light brown colour spots (Figure 4A).
2.	Pale-olive colour body speckled with numerous dark spots (Figure 4B).

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